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Growth and Metabolic Responses of Saccharum Species in Relation to Flooding.

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ABSTRACT

Some growth and metabolic responses of different clones of Saccharum in relation to their flood-tolerance were examined. Clones grown in soil subjected to flooding demonstrated their ability to produce water roots as a means of morphological adaptation to flooding. Significant differences were observed among the clones with respect to the quantities of water roots produced, but all the clones were visually tolerant to flooding. Clones representing Saccharum spontaneum followed by the commercial hybrid produced the maximum quantities of water root under the flooded condition. The clones of Saccharum barberi and Saccharum robustum produced the least quantities of water root.

Artificially imposed anoxia under nutrient culture provided a basic screening technique for the tolerant and intolerant clones. The tolerant clones suffered only a minimal reduction in root growth ($\leq$40%) and stalk elongation (41-53%) whereas the intolerant clones suffered greater reductions in root growth (>50%) and stalk elongation (80-98%) under anoxia. Intolerant clones tended to cease stalk elongation and root growth beyond 20 days of anoxic treatment while the tolerant clones continued to grow throughout the experimental period. Both inter- and intraspecific differences in growth parameters were observed with the clones tested.

Among the metabolic parameters, the activity of the enzyme alcohol dehydrogenase (ADH) appeared to hold some merit for

identifying the intolerant clones. Although two different trends of responses were obtained with the tolerant clones viz., Coimbatore, CP 65-357, and NG 77-59, this enzyme showed a consistently high level of activity in the intolerant clones viz., NG 77-160, Cavengerie and D-74. The activity of the enzymes, malate dehydrogenase, lactate dehydrogenase and peroxidase did not show any metabolic significance in relation to flooding tolerance of the clones tested. A low level of pyruvate decarboxylase activity was detected only at 3 days of anoxia. Of the metabolites, the concentration of ethanol appeared to complement the response characteristics due to ADH. Other metabolites viz., malate, sucrose, glucose and fructose, did not show any consistent changes that could be correlated with tolerance or intolerance of the different clones tested; however, there were consistently higher levels of ethanol, malate and sugars under anoxic conditions.

PART I. DEVELOPMENTAL CHANGES IN SUGARCANE UNDER FLOODING OR ANOXIA

INTRODUCTION

Many researchers have investigated the adverse effect of flooding on the growth of a wide range of plant species viz., Tobacco (Kramer and Jackson, 1954; Williamson, 1970; Willey, 1970), Sunflower (Kawase, 1974), Corn (Drew et al., 1980; Konings, 1982), Pea (Healy and Armstrong, 1972), Tomato (Bradford and Hsiao 1982; Reid and Crozier, 1971), Wheat (Trought and Drew, 1980), and many other wetland species (Lambers and Steingrover, 1978; Teal and Kanwisher, 1966). It has been established that plants tolerant to flooding often possess morphological adaptations that enable them to overcome the deleterious effects of flooding or anoxia. Hook and Scholtens (1978) described morphological adaptation as the ability of a plant species to survive flooding by producing secondary roots or adventitious roots on the submerged stem.

Crawford (1976) reported that in flood-tolerant species Salix, a limited amount of aeration was supplied to the roots by marked development of lenticels at the base of the trunk. Jackson (1955) reported that the formation of adventitious roots reduced the flooding injury in tomato plants. Tang and Kozlowski (1982) reported that flooding drastically inhibited shoot growth, root growth, and caused root decay in Betula papyrifera usually considered a flood-intolerant plant. Kramer (1951) reported that the degree of injury was the least in sunflower which was the first species to develop adventitious roots, and the greatest in tobacco found to be the slowest to develop

adventitious roots. Kramer and Jackson (1954) also observed cessation of stem elongation in tobacco (flood intolerant) accompanied by epinasty and yellowing of leaves and finally death.

Sartoris and Belcher (1949), during the 1947 natural flooding in southern Florida, reported that the ability of the different species of Saccharum to produce primordial water roots at submerged joints largely determined their growth and survival under prolonged flooding.

No systematic research approach on the growth responses of sugarcane under flooding has yet been undertaken. Recently, the need for selecting sugarcane genotypes with superior tolerance to flooding has been emphasized for the southern regions of the United States (Anon, 1974). Reports also indicated that high water table and excess water were largely responsible for low yield of sugarcane in Louisiana. Such reports also emphasized the need for improved subsurface drainage for higher productivity of sugarcane (Carter, 1976; Carter and Floyd, 1974; Lal and Patrick, 1965). For the future, sugarcane varieties with increased flooding-tolerance are expected to contribute a great deal to the overall production of sugarcane in Louisiana and will allow further expansion of this crop without the need for complete drainage.

The present investigation was undertaken to examine the effects of flooding or anoxia on some growth responses of five different species of Saccharum each represented by a suitable clone, and a commercial hybrid (vide the appendix showing the classification of the clones). The study was further extended to cover some S. officinarum clones to investigate intraspecific differences.

In order to accomplish the overall objectives, three separate sets of experiments were conducted:

A. Flooding-tests with Saccharum species.

This experiment, with the representative Saccharum species and a commercial hybrid, was designed to examine the relative ability of the different representative clones to produce primordial water roots upon submergence of the main root system (in soil) and part of the stem A second flooding-test was also conducted to determine the relative contribution of the main root (in soil) and the water root to growth (stalk elongation) under flooding. The experiments included the clones Coimbatore, Katha, Chunnee, NG 77-160, D-74 and CP 65-357.

B. Growth studies with Saccharum species under Anoxic Nutrient Culture.

This experiment was designed to examine the effect of anoxia (developed by flushing nitrogen through the nutrient solution; Eh ca 205 mV) on stalk elongation rate, root production, and foliar symptoms of injury of the representative Saccharum species and the commercial hybrid as mentioned under A, grown in anoxic nutrient solution.

C. Growth studies with Saccharum officinarum clones under Anoxic Nutrient Culture.

This experiment was conducted as a follow-up of experiment B to examine the effects of anoxia on stalk elongation rate, root and shoot production, and visual symptoms of injury to some representative S. officinarum clones, viz., Cavengerie, D-74, NG 77-43, and NG 77-59. Commercial hybrid, CP 65-357, was also included for comparison.

LITERATURE REVIEW

The first successful controlled hybridization of sugarcane by Fairchild in 1708 opened a new era in the breeding history of this industrial cash crop, and provided the basic inspiration for "crossing the best with the best and hoping for the best" (Stevenson, 1965; Alexander, 1973). One of the first attempts to improve sugarcane via controlled crosses was performed in Java during the 1880's as a consequence of the 'sereh-disease' outbreak on Black Cheribon, the major variety in cultivation at that time (Alexander, 1973, Jeswiet, 1929). Roach (1971) pointed out that the outbreak of this disease served as a primary stimulus for the hybridization of noble canes commenced by Soltwedel in Java in the late 1880's utilizing the wild species, Saccharum spontaneum, and its naturally occuring hybrid, Kassoer. This effort culminated in 1921 with the production of the interspecific hybrid, POJ2878, known as the 'wonder cane' in the sugarcane world (Stevenson, 1965).

As time passed, the priorities for breeding sugarcane were continually tailored to meet the local needs which embraced disease and pest resistance, high sugar, high tonnage, cold tolerance, milling quality and other agronomic characters. At the present, one priority being considered is flooding tolerance. While determining the research needs of sugar crops for the southern regions of the United States, a 'Joint Task Force' emphasized the need for evaluating sugarcane germ plasm tolerant or resistant to flooding. The report also

stressed the need for evaluating the potential of the wild Saccharum clones and their relatives before they disappear from their natural habitat due to the encroachment of civilization (Anon, 1974). In many areas of the world where socio-economic and other factors have rendered sugarcane to compete with other crops, and where only the marginal land with poor drainage and periodic inundation is available for such a crop of great industrial value, the need for flood-tolerant varieties does exist.

To produce varieties with superior tolerance to flooding, the primary emphasis is obviously placed on the exploitation of the available genotypes endowed with desired qualities and characteristics. Unfortunately, even after a few centuries of our dependence on the noble canes (Saccharum officinarum) and wild types (S. spontaneum and S. robustum) contributing to the ancestry of modern varieties, there is a lack of information on their reaction to flooding. This is also true for S. sinense and S. barberi which have been used in commercial cultivation for manufacturing sugar (Stevenson, 1965). Only sparse information indicates that some wild types can adapt to adverse ecological situations including flooding (Clements, 1980; Alexander, 1973).

During the late 1920's and 1930's, the cumulative knowledge on wild canes was greatly advanced, largely as a result of expeditions to their primitive habitats for the purpose of conserving genotypes. It is believed that apart from the qualities of ecological adaptability, S. robustum representing a broad natural range has played a great role in the development of the S. officinarum group (Alexander, 1973). The S. robustum habitat is often reported as being along river banks where

it survives periodic inundation. One of the unusual characteristics of S. robustum in the natural habitat is that is forms long stolons up to 60 feet which grow out over the mud flats of the rivers. During a flood these stolons float on the surface of the water. Roots and sometimes shoots develop from stolons in the natural habitat, but seldom in plantings removed to other places (Alexander, 1973).

At this point, it is deemed appropriate to briefly introduce some basic characteristics of flooded soil as it relates to plant adaptation and survival.

The characteristics of flooded soil have been adequately examined by many researchers (Armstrong, 1978; Gambrell and Patrick, 1978; Stolzy and Fluhler, 1978; Ponnamperuma, 1955). Regardless of soil types, certain properties are common to flooded soil. Foremost among these is oxygen deficiency. The diffusion coefficient of oxygen is very high in air and is estimated to be 2.05×10^{-2} cm^2 s^{-1} @ 23C while that in water is 2.267×10^{-5} cm^2 s^{-1}. This means that under flooded condition, diffusion occurs at about 1/1000 the rate of gaseous diffusion (Armstrong, 1978). Complete anaerobiosis may develop within a few hours of flooding. Aerobic organisms use up the oxygen present in the soil pore space and become quiescent or die while facultative anaerobes followed by obligate anaerobes take over the decomposition of organic matter using oxidized soil components other than oxygen as electron acceptors in their respiration (Gambrell and Patrick, 1978; Ponnamperuma, 1955). A shift from aerobic to anaerobic metabolism takes place in widely differing soil at an oxygen concentration of less than 0.1 ppm in soil solution (Stolzy and Fluhler, 1978). The decrease in redox potential (Eh) is another

important characteristic of flooded soil. In oxidized soil containing molecular oxygen, the redox potential is reported to range from about +400 to +700 mV. In submerged soils, the redox potential may go down to -400 mV i.e., strongly reduced state (Gambrell and Patrick, 1978). The third important characteristic of flooded soil is the sequential reduction of the inorganic redox system. Nitrate reduction is reported to occur around 200 mV. Nitrate reduction may begin before complete removal of O_2, but complete NO_3^- reduction probably does not does not occur until all O_2 is depleted. The redox potential (Eh) at which oxygen depletion take place ranges between 320 to 340 mV for most soils. Next to NO_3^-, manganese (Mn^{4+}) is reduced to the more soluble form (Mn^{2+}) as Eh decreases to approximately 200 mV. Around 120 mV, ferric iron (Fe^{3+}) is reduced to Fe^{2+} form, while sulfate reduction occur around -150 mV. After sulfate reduction, CO_2 is reduced to methane between -250 to -300 mV at which range maximum population of methane producing bacteria exists (Gambrell and Patrick, 1978).

Plants vary widely in their response to low-oxygen status. For most plants, root growth will be limited in soil with fewer than 10% air-filled pore spaces. Crops like rice can grow with no soil oxygen while other plants are very sensitive to low oxygen level (Meek and Stolzy, 1978).

The present status of knowledge on plants' adaptation to flooding reveals at least two important aspects:

(a) Some plants can survive flooding by adaptation viz., morphological, anatomical, and metabolic

adaptations; and, such adaptations may or may not be mutually exclusive.

(b) Populations within the same species may be endowed with tolerance to flooding.

Hook and Scholtens (1978) reported that certain tree species have evolved with adaptation which enable them to cope with flooded environments. Buttress and knee development on bald cypress trees in swamps are two examples. When the lower trunk and root of tree species are inundated by water, normal gas exchange through the bark is impeded. Under this condition the cambium, actively growing root tips, and other vital tissue must receive their aeration by transport of oxygen from plant parts above the water level. They further noted that periodic or continuous flooding of the stem of certain tree seedlings results in the proliferation of lenticels on the flooded stem and roots. Depending on species, lenticels may play dual role in aeration. Oxygen from the atmosphere can diffuse into the plants through the lenticels and internal ethanol, acetaldehyde, the dominant products of anaerobic metabolism, diffuse out through the same lenticels. In poplar, however, oxygen enter the lenticel but no internal metabolites are excreted (Chirkova and Gutman, 1972). Crawford (1976) reported that in the flood-tolerant species Salix, a limited amount of aeration is supplied to the roots by the marked development of lenticels at the base of the trunk. Lenticels allow the upward diffusion and removal of toxic substances such as ethanol, from the roots, as well as the downward diffusion of oxygen.

Besides structural modification to stems, deWit (1978) reported structural modification of roots under oxygen stress. There are some

plants viz., maize, wheat, and barley that normally grow well in aerated soil, but when subjected to flooding or oxygen depletion, they are able to form air space or lacunae in the root cortex. To explain the mechanism of lacunae formation, some researchers hypothesize that anoxia initiates a series of reactions causing rapid breakdown of cytoplasm and of the protein in the cell wall leading to the formation of lacunae in the cortex. Others believe that air space formation is a process controlled by IAA. As the peroxidative inactivation of IAA transported from shoot to the roots is an oxygen requiring process, it seems likely that oxygen deficiency could lead to a higher IAA content of the roots. Ethylene is also considered another factor responsible for air space formation in roots. deWit (1978), in trying to pull the available information on this particular aspect of anatomical adaptation, concluded that lack of oxygen locally starts a series of biochemical processes that lead to death and destruction of clusters of cells. These processes and their interrelations are not sufficiently understood, but IAA, ethylene and calcium appear to play important roles in the formation of lacunae or air cavity in roots (deWit, 1978, Coutts and Armstrong, 1976; Hook et al., 1972). This aspect of plant adaptation has been investigated by a number of other workers (Armstrong, 1967, 1971; Teal and Kanwisher, 1966; Drew et al., 1980; Konings, 1982; Yamasaki and Saeki, 1976; and John, 1977).

Coutts and Armstrong (1976) reported that some herbacious plants survive on waterlogged condition by exploiting only the oxygenated soil layer or sediment. In some cases, adventitious roots can be produced which grow horizontally within the saturated horizon along a tolerable redox plane, with ascending lateral roots. Some

flood-tolerant Salix species readily produce adventitious roots in water-logged soils. Kawase (1974) working on sunflower, was able to correlate ethylene concentration and flooding injury including epinasty and chlorosis. Anatomical observation also revealed increased intercellular air spaces in the cortex. He concluded that under flooded condition, increased ethylene concentration was largely responsible for flooding damage symptoms.

Kramer (1951) hypothesized that lack of oxygen near the waterline may interfere with downward translocation of carbohydrates and auxin. Hence their accumulation near the flood line would stimulate hypertrophy of the tissue and water root development below the waterline.

Crawford (1978a) noted that many wetland species, despite abundance of water in the soils, exhibit many of the xeromorphic features characteristic of drought-adapted plants. The reduction of leaves in Juncus and the hairy folded leaves of the crossleaved heath (Erica) are examples.

It was the opinion of Barclay and Crawford (1982) that flooding tolerance is likely to depend on a number of adaptations both physical and metabolic with the importance of each varying between different species of wetland plants. They observed interspecific differences in the ability of seedlings and rhizomes to survive under prolonged and strict anaerobiosis.

Levitt (1980) recognizes flooding stress as secondary stress, and states that since flooding replaces gaseous air by liquid water, the immediate gas stress in the form of O_2 deficit, CO_2 excess and ethylene excess comes into operation.

Gomes and Kozlowski (1980) working on flood-tolerant Fraxinus pennsylvania, reported the formation of hypertrophied lenticels and production of adventitious roots on submerged portions of the stem above the soil line. They noticed that after 15 days of stomatal closure as a result of flooding, stomata began to reopen about the time when adventitious roots began to form.

Hook and Scholtens (1978) defined morphological adaptations as the ability of (i) plant species to produce secondary roots to survive prolonged flooding, (ii) seedlings to regenerate new secondary roots from primary roots, and (iii) seedlings to develop adventitious water roots on the submerged stem. They further reported that many woody species develop adventitious or water roots under the influence of flooding at or just below the surface water level. Such roots are thought to aid the species in flood-tolerance.

Reporting the role of adventitious roots in tomato plants, Jackson (1955) showed that the formation of adventitious roots did not completely prevent the flooding injury, but leaf epinasty was less and shoot growth was greater. Resumption of shoot growth occured when the adventitious roots were allowed to develop after flooding, while shoot growth was practically stopped when such roots were removed.

Fitter and Hay (1981), after reviewing flooding stresses and adaptations, concluded that flood-tolerant plants must possess some sort of anatomical, morphological, biochemical and physiological adaptations leading to avoidance or amelioration of the many unfavorable features of flooded soils. They further concluded that anatomical and morphological features are responsible for improving the transport of oxygen to respiring roots. Biochemical features permit

prolonged anaerobic glycolysis while the physiological mechanisms account for exclusion of phytotoxic substances in the rhizosphere.

In part I of this study, I will examine some growth parameters which may be useful in selecting Saccharum genotypes with superior tolerance to flooding. Part II is devoted to metabolic adaptations to anoxia.

MATERIALS AND METHODS

Collection and Multiplication of Plant Materials

Sugarcane clones representing five different species of Saccharum viz., S. spontaneum (represented by clone Coimbatore), S. sinense (Katha), S. barberi (Chunnee), S. officinarum (D-74), S. robustum (NG 77-160) and commerical hybrid (CP 65-357) were collected from the USDA Sugarcane Field Laboratories, Houma, Louisiana in September, 1981. Stalks were heat treated in a controlled-temperature water bath at 50.5C for 20 minutes for the first day and 3 hours for the second day according to Dr. Benda, Sugarcane Pathologist, USDA, Houma (Personal Communication). Single-bud setts (segments), prepared from the stalks, were then planted in flats containing Jiffy Mix Plus (Jiffy Products Co.). Six-week old seedlings (settlings) were then transplanted to 32 cm x 24 cm diameter plastic pots for multiplication to ensure enough materials for subsequent experiments.

For the experiment with Saccharum officinatrum clones, a separate lot of heat treated plant material was collected from the World Sugarcane Collection at Florida in December, 1982 through the assistance of Dr. J. D. Miller, Research Geneticist, USDA Sugarcane Field Station, Canal Point, Florida. These materials included four clones of S. officinarum viz., NG 77-43, NG 77-59, Cavengerie, and D-74. The commercial hybrid, CP 65-357, included in the test for comparison, was collected at St. Gabriel Experiment Station, LSU. Single-bud segments were transplanted in Jiffy Mix Plus as before for seven weeks, and then seedlings were transplanted to pots (20 x 30 cm

diameter) containing a modified Hoagland solution (Johnson et al., 1957; Epstein, 1971).

Flooding Test with Saccharum Species

Six week old plants raised from single-bud segments were transplanted to 17.5 x 15 cm diameter plastic pots (one plant per pot) containing soil filling the pot up to 15 cm depth. Plants were watered everyday and fertilized every two weeks with liquid commercial fertilizer containing 12-6-6 NPK at the rate of 20 ml per gallon of water, and were raised through the tiller initiation stage. At this time 50% of the plant population (8 pots) of each species including the commercial hybrid was immersed in water contained in 32 x 25 cm diameter plastic pots. The immersed pots with the plants had an overlying water table of about 15 cm and was maintained as such until the end of the six-week test. A separate flooding-test with identically grown plants was conducted for two months using 56 cm x 45 cm diameter plastic pots allowing an overlying water table of about 35 cm. In this experiment, the primordial water roots (PR) were allowed to develop for one month of flooding and the stalk elongation rate was recorded during this period. This represented control-plants with the main root in soil (MR) and the PR intact. Then in one set of plants (4 plants per species) the MR was detached leaving the PR undisturbed, while in another set of plants, the PR was removed leaving the MR intact. The growth (stalk elongation) rate under both conditions was recorded. Both the tests were conducted in a glass house with partial control of temperature ranging from 25C to 35C.

Redox Potential Measurement

Redox Potential (Eh) of the flooded soil was measured by using a platinum electrode and a saturated calomel reference electrode (SCE). The platinum electrode was placed about 4 cm deep in the immersed pot. Measurements were made by inserting the SCE in the pot approximately 5 cm from the platinum electrode. To calculate the Eh, the potential of the Calomel electrode against a standard hydrogen electrode (i.e., +242 mV) was added to the measured potential (Sims, 1981) and results expressed in mV (millivolts).

To follow the Eh of the nutrient solution, the same procedure as above was followed except that the platinum electrode was held in the nutrient solution instead of the soil.

Determination on Primordial Water Roots

After six weeks of flooding, the immersed pots were removed. Shoots with primordial roots formed at each submerged joint, were detached at the soil level, and such roots were collected and sampled separately for each pot. The roots were washed with tap water and oven dried at 100C to a constant weight.

The Nutrient Culture Set-up

The experimental set-up was essentially comprised of 15 liter capacity 20 x 30 cm diameter plastic pots with styrofoam lid serving as substratum for the plants held in small 50 ml polyethylene beakers which were perforated to allow the root to spread into the solution. One plant was placed in each such beaker and held upright with the help of a suitable Dispo-plug (T-1387, Scientific Products Co.). Each pot accomodated sixteen plants. To give additional sealing, a weather

strip cord (Mortell Co., Kankakee, Ill.) was used along the border of the styrofoam lid. Full strength Hoagland solution as modified by Johnson et al. (1957) was used throughout the experiment. The solution was replaced every week.

Following transplanting of seven week old plants to the nutrient solution, the entire population of the plants under the experiment was equilibrated by raising them under identical aerated conditions for three weeks and, thereafter, 50% of the plants were subjected to anoxic treatment by continuously flushing with nitrogen gas (pure) at the rate of ca. 130 ml per min from a cylinder. The plants earmarked for the aerated treatment (control) continued to be bubbled with air at the rate of ca. 1000 ml per min. Airstones (#57228-600, Central Scientific Co.) were used in each bucket for even distribution of air or nitrogen gas in the nutrient solution. The whole set-up of the nutrient culture was housed in a green house in which temperature was maintained at 30C ± 3C, and natural day light ranging from 700 to 900 $\mu Es^{-1}m^{-2}$ was supplemented with artificial light for 3 hr in the morning and 3 hr in the evening.

Measurement of Growth (Stalk Elongation) Rate

Eight plants per pot were marked at the base with a marking pen and their initial height (height at 0 day) from the base to the TVD (Top most Visible Dewlap) was recorded. The 0-day represents the day of starting the anoxic treatment by flushing with nitrogen. Plant height readings were recorded prior to harvesting the roots at 3, 10, 20 and 30 days. The growth data upto 20 days is the average of eight plants while that of 30 days is of four plants per pot left for the final harvesting under both aerated and anoxic conditions.

Determination on Root Production

A composit sample of roots from four plants harvested for enzyme activity and metabolites determinations were collected and placed in paper bags of known oven dry weight. The roots were oven dried at 100C until constant weight. To this weight was also added the dry weight (derived from the fresh weight) of the roots sampled for determinations of enzyme activity and metabolites.

Determinations of Shoot Production

Plants cut at the base were collected in paper bags of know oven dry weight, and oven dried at 100C until constant weight.

Statistical

To determine the effect of anoxia over aerated control, pairwise comparisons with a prior hypothesis were made with a one-tailed t-Test. In some cases, standard error was calculated to provide an estimate of variance. LSD (Least Significant Difference) values were worked out where a significant difference was obtained by the F-test (Steel and Torrie, 1980). An α-level of 0.05 was selected as the criterion for declaring significant difference.

RESULTS

A. Flooding - Test With *Saccharum* species.

Effect of Flooding on Soil Reduction and Primordial Water Root Production

Redox potential (Eh) measurements of the flooding-test are presented in Table 1. The immersion of the pots in water resulted in a gradual decrease in Eh indicative of the depletion of oxygen and reduction of other inorganic redox systems.

All the clones under study produced primordial water roots under flooded conditions although there was a significant difference among them in terms of the quantity of roots produced (Table 2). Chunnee (representing *S. barberi*) and NG 77-160 (*S. robustum*) tended to produce the least quantity of primordial water roots while clone Coimbatore (*S. spontaneum*) produced the most. Commercial hybrid, CP 65-357 also demonstrated a high potential in producing water roots. D-74 (*S. officinarum*), Katha (*S. sinense*) appeared to be of intermediate category in the production of primordial water roots in response to flooding.

Table 3 shows the relative importance of the main root system in the soil (MR) and the primordial water root (PR) of the *Saccharum* species and the commercial hybrid subjected to a two-month flooding-test. The data indicates that under submerged conditions, the water roots (PR) contribute the most to stalk elongation growth. There was a reduction in growth in all the clones when the main root system (MR)

Table 1. The changes in redox potential (Eh) of the immersed pots during a six-week flooding-test.

Day(s) after flooding	Eh* (mV)
1	122 (± 12)
3	-158 (± 10)
7	-229 (± 6)
35	-290 (± 4)

* Expressed in millivolt at pH 7.0, the values are the means (± standard error) of at least six observations.

Table 2. Production of primordial water roots by the Saccharum species and the commercial hybrid grown in soil-filled pots, upon immersion in water for six weeks.

Species	Root dry wt. (g plant^{-1})*
Saccharum spontaneum (Coimbatore)	3.40
Saccharum sinense (Katha)	1.65
Saccharum barberi (Chunnee)	0.57
Saccharum robustum (NG77-160)	0.73
Saccharum officinarum (D-74)	1.21
Commercial hybrid (CP 65-357)	2.52
LSD (0.05)	0.31

* Values are means of eight plants.

Table 3. The effect of removing the main root system (MR) or primordial water root (PR) on stalk elongation during a two-month flooding test with the Saccharum species and the commercial hydrid.

Species	Elongation Rate (cm day^{-1})*		
	(+MR, +PR)	(-MR, +PR)	(+MR, -PR)
Saccharum spontaneum (Coimbatore)	1.87	1.72	0.93
Saccharum sinense (Katha)	1.48	1.01	0.63
Saccharum barberi (Chunnee)	0.96	0.68	0.04
Saccharum robustum (NG77-160)	0.40	0.11	0.02
Saccharum officinarum (D-74)	0.73	0.42	0.20
Commercial Hybrid (CP 65-357)	1.47	0.63	0.38
LSD (0.05)	0.26	0.11	0.09

* Values are means of four plants.

was detached but its overall effect on growth was not as severe as when the PR was excised with the MR still undisturbed. Chunnee (S. barberi) and NG 77-160 (S. robustum) showed only 4% and 5% elongation growth respectively in the absence of PR indicating their possible dependence on such roots to a large extent.

B. Growth Studies With Saccharum Species Under Anoxic Nutrient Culture.

Effect of Anoxia on Stalk Elongation

The effect of anoxia on stalk elongation of the six representative clones including the commerical hybrid, CP 65-357 is presented in Table 4. Anoxia significantly decreased the stalk elongation in all the clones as compared to their aerated controls, but the degree of reduction varied from clone to clone. The maximum reduction (97-98%) in stalk elongation due to anoxia was observed with D-74, NG 77-160 and Chunnee (Table 5 and Figures 1 and 2) after 20 days of growth under anoxic nutrient culture. Based on the visual symptoms (viz., leaf chlorosis, desiccation, etc.) of anoxia-injury, and %-reduction in stalk elongation, these clones appeared to be intolerant. Considering visual symptoms alone, however, Chunnee appeared to be of intermediate tolerance (Table 5). CP 65-357 and Coimbatore were tolerant to anoxia or flooding. However, they suffered a reduction in stalk elongation under anoxia to the extent of 41% and 46% respectively. Katha appeared visually close to the tolerant clones, but, had a 71% reduction in stalk elongation (Table 5) after 30 days of anoxic treatment.

Table 4. Stalk elongation rate of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2/]	Elongation Rate (mm day^{-1})[1/]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	3.43	4.33	7.43	9.86	6.26
	Anoxic	3.31	3.27	4.77	4.23	3.89
S. sinense (Katha)	Aerated	2.69	3.23	6.88	5.32	4.53
	Anoxic	2.67	2.85	2.75	0.90	2.29
S. barberi (Chunnee)	Aerated	3.27	4.18	6.25	6.05	4.94
	Anoxic	1.49	0.60	0.29	0.00	0.59
S. robustum (NG77-160)	Aerated	1.59	1.46	1.82	1.45	1.58
	Anoxic	1.45	0.40	0.06	0.00	0.47
S. officinarum (D-74)	Aerated	2.29	3.02	3.15	1.60	2.51
	Anoxic	0.75	0.44	0.00	0.00	0.30
Commercial Hybrid (CP 65-357)	Aerated	3.22	3.27	3.96	3.40	3.46
	Anoxic	3.15	2.16	1.98	1.77	2.26

[1/] Values are means of at least eight plants.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 5. Relative tolerance ratings of different clones to experimental flooding or anoxia based on visual symptoms of injury and reductions in root production and stalk elongation rate. Clones are listed in descending order of tolerance under each category of rating.

Visual Symptom[1/]		Root Production[2/]		Stalk Elongation[3/]	
Coimbatore	(1)	Coimbatore	(31)	CP 65-357	(41)
CP 65-357	(1)	CP 65-357	(36)	Coimbatore	(46)
NG77-59	(1)	NG77-59	(40)	NG77-59	(53)
Katha	(2)	Katha	(49)	Katha	(71)
Chunnee	(3)	NG77-160	(53)	NG77-43	(74)
NG77-160	(4)	Cavengerie	(58)	Cavengerie	(80)
Cavengerie	(4)	D-74	(59)	Chunnee	(97)
NG77-43	(4)	NG77-43	(60)	NG77-160	(98)
D-74	(5)	Chunnee	(71)	D-74	(98)

[1/] Ratings on visual symptoms (viz., leaf chlorosis and desiccation) of injury are based on a 0-5 scale (ranging from 0 = No injury to 5 = Very severe injury) after 20 and 30 days of anoxic treatment. Values in the parentheses indicate injury ratings.

[2/] Rating on root production is based on %-reduction of root production under anoxia as compared to the control. Values in the parentheses indicate the mean values on %-reduction of root production with 20 and 30 days data.

[3/] Rating on stalk elongation is based on %-reduction of stalk elongation rate under anoxia as compared to the control. Values in the parentheses indicate the mean values on %-reduction of stalk elongation rate with 20 and 30 days data.

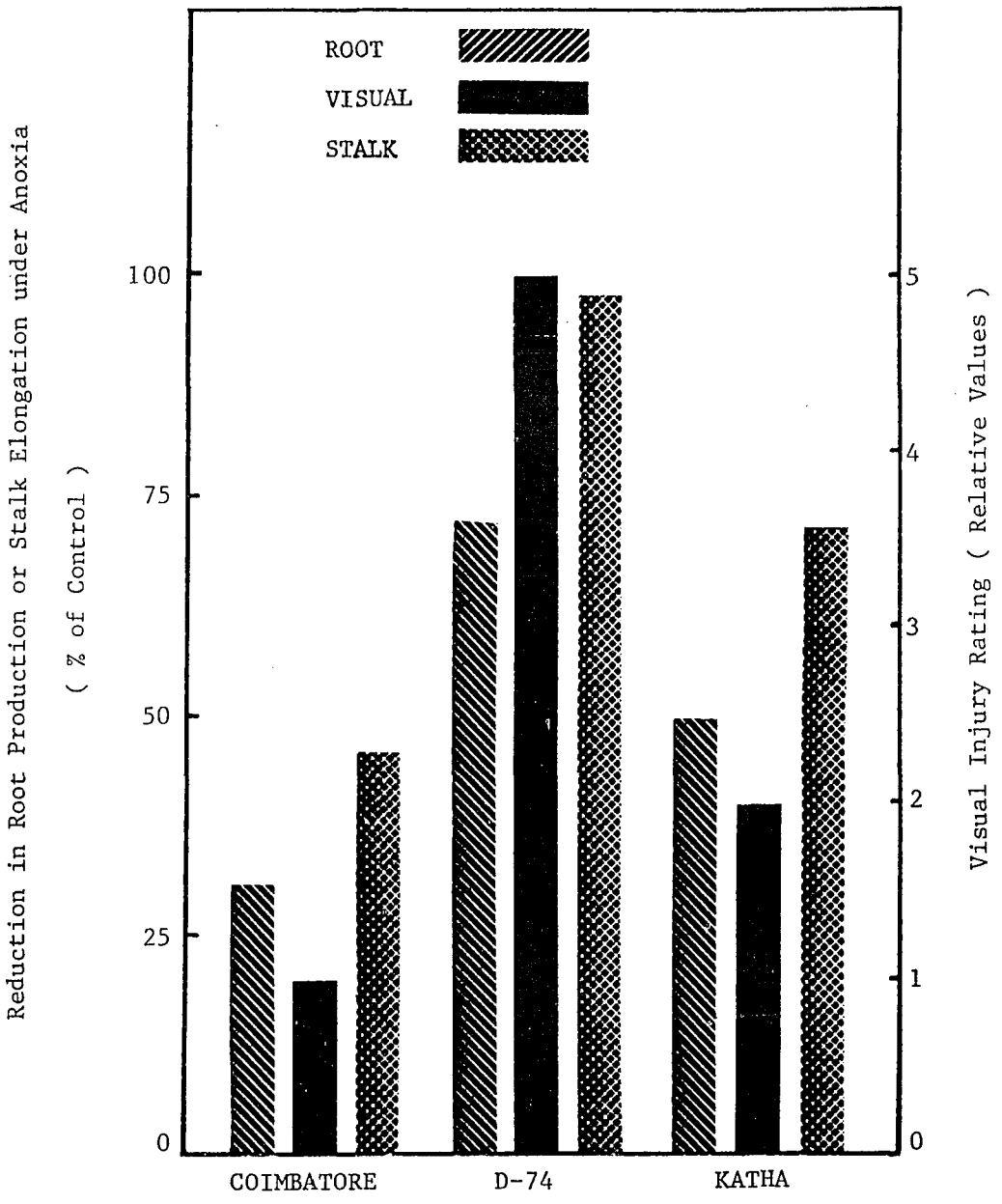

Figure 1. Root production, stalk elongation rate, and visual tolerance of three Saccharum clones, viz., Coimbatore (tolerant), D-74 (intolerant) and Katha (moderately tolerant), as affected by 30 days of anoxia. Higher values on visual injury rating indicate poorer tolerance to anoxia or flooding.

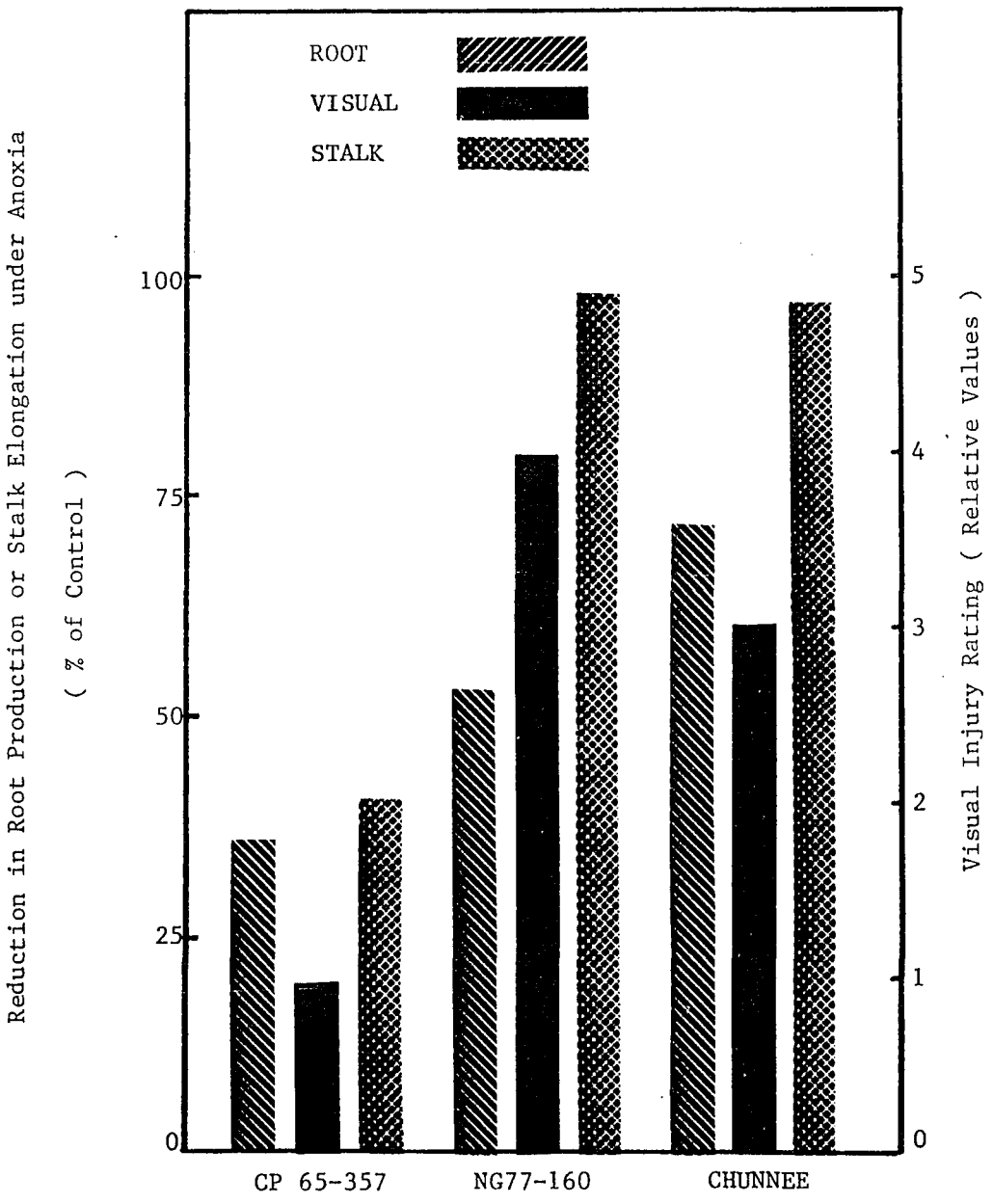

Figure 2. Root production, stalk elongation rate, and visual tolerance of three Saccharum clones viz., CP 65-357 (tolerant), NG77-160 (intolerant) and Chunnee (moderately intolerant) as affected by 30 days of anoxia. Higher values on visual injury rating indicate poorer tolerance to anoxia or flooding.

Effect of Anoxia on Root Production

Table 6 shows the effect of anoxia on root production by the different representative clones including the commercial hybrid. Anoxia significantly decreased root production in all the clones when compared to the aerated control. In Table 5, the different clones are arranged in the descending order of their root producing ability under anoxia. The visually tolerant clones viz., Coimbatore, CP 65-357 demonstrated the least reduction (31% and 36% respectively) in root production under anoxic condition. Chunnee suffered the maximum reduction in root production under anoxia. Katha appeared to be of intermediate category with 49% reduction in root production. NG 77-160 considered intolerant based on visual symptoms and stalk elongation rate, also appeared to be of intermediate category in the light of its ability to produce roots under anoxic conditions.

An examination of data (Table 6) further reveals that under anoxia, tolerant clones viz., Coimbatore, CP 65-357 continued to maintain a fairly good rate of root growth while intolerant ones viz., NG 77-160 and D-74 failed to increase their root mass beyond 10 days of anoxic treatment.

C. Growth Studies With *Saccharum officinarum* Clones Under Anoxic Nutrient Culture.

Effect of Anoxia on Stalk Elongation

The results on the stalk elongation of different clones including the commercial hybrid, CP 65-357, as affected by anoxia are reported in Tables 5 and 7. Anoxia caused significant reduction in stalk elongation as compared to the aerated control. The maximum reduction

Table 6. Root production by the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2/]	Root Dry wt. (mg plant^{-1})[1/] Duration of Treatment (Days) 3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	267	390	723	1163	636
	Anoxic	226	280	506	790	450
S. sinense (Katha)	Aerated	307	665	1517	2925	1353
	Anoxic	225	358	692	1602	727
S. barberi (Chunnee)	Aerated	349	645	2260	2775	1507
	Anoxic	245	283	525	937	497
S. robustum (NG77-160)	Aerated	272	430	689	885	569
	Anoxic	236	320	344	385	321
S. officinarum (D-74)	Aerated	309	432	782	989	628
	Anoxic	262	265	335	348	302
Commercial Hybrid (CP 65-357)	Aerated	648	1104	2325	3102	1795
	Anoxic	544	822	1495	1825	1171

[1/] Values are means of eight plants

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 7. Stalk elongation rate of the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2]	Elongation Rate (mm day^{-1})[1]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
Cavengerie	Aerated	1.62	1.58	1.50	1.40	1.52
	Anoxic	1.52	0.90	0.37	0.20	0.75
D-74	Aerated	1.91	2.25	2.30	1.50	1.99
	Anoxic	1.01	0.35	0.14	0.00	0.37
NG77-43	Aerated	2.06	2.31	2.10	2.00	2.12
	Anoxic	1.95	1.23	0.75	0.30	1.06
NG77-59	Aerated	4.04	4.41	5.04	4.17	4.41
	Anoxic	2.97	2.71	2.05	1.94	2.42
CP 65-357	Aerated	3.82	3.69	3.48	2.97	3.49
	Anoxic	3.00	2.49	2.21	2.03	2.43

[1] Values are means of eight plants except for the last period (21-30 days) which represent four plants.

[2] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

in stalk elongation was evident in D-74 (98%) Covengerie (80%) and NG 77-43 (74%), all of these clones being visually rated intolerant (Table 5 and Figure 3). The clone NG 77-59, found tolerant on visual rating, had the least amount of reduction (53%) in stalk elongation under anoxic treatment.

Effect of Anoxia on Shoot Production

Table 8 shows the shoot dry matter production by some S. officinarum clones and the commercial hybrid, CP 65-357 under aerated and anoxic conditions. Significant reduction in shoot dry weight occured under anoxic treatment. There was an increasing trend of shoot dry matter production in all the clones even under anoxic conditions. On the average, the commercial hybrid produced the highest quantity of shoot dry matter under anoxia followed by NG 77-59 and D-74, the latter being intolerant to flooding. The clones Covengerie, D-74 and NG 77-43 grew poorly under both the aerated and anoxic conditions.

Effect of Anoxia on Root Production

Table 9 shows data on root dry matter production by some S. officinarum clones and the commercial hybrid, CP 65-357 under both aerated and anoxic treatments. Anoxia caused significant reduction in root production as compared to the aerated control. The relatively tolerant clones CP 65-357 and NG 77-59 maintained a progressive increase in root production under anoxia throughout the experimental period. The intolerant clones, viz., Cavengerie, D-74, and NG 77-43 had greatly reduced root growth beyond 10 days of anoxic treatment.

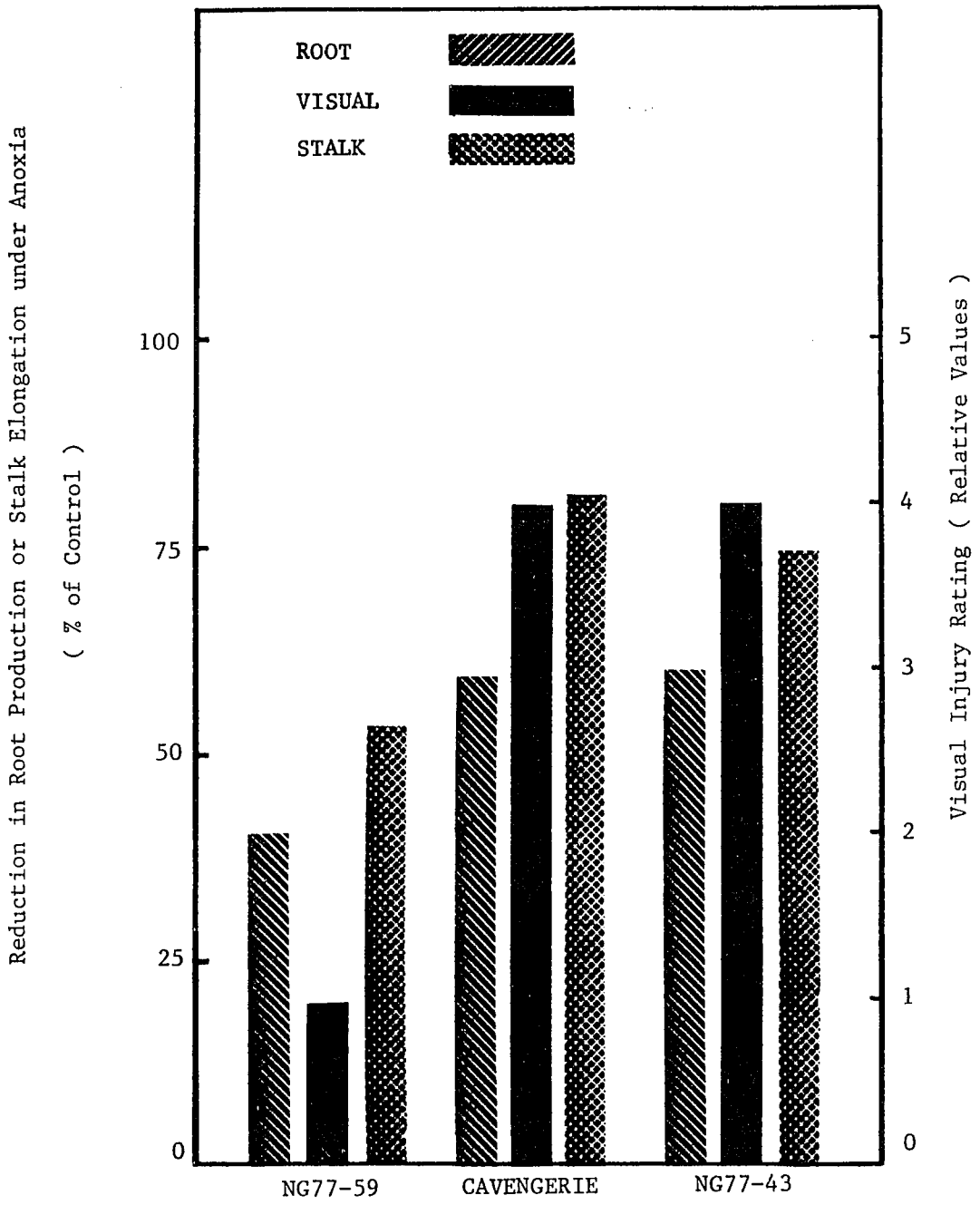

Figure 3. Root production, stalk elongation rate, and visual tolerance of three Saccharum clones viz., NG77-59 (tolerant), Cavengerie (intolerant) and NG77-43 (moderately intolerant) as affected by 30 days of anoxia. Higher values on visual injury rating indicate poorer tolerance to anoxia or flooding.

Table 8. Shoot dry matter of the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2/]	Shoot Dry wt. (g plant^{-1})[1/]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
Cavengerie	Aerated	0.96	1.13	1.22	2.79	1.52
	Anoxic	0.80	0.91	0.96	1.63	1.07
D-74	Aerated	4.38	5.06	5.89	6.69	5.50
	Anoxic	4.03	4.22	4.50	5.50	4.56
NG77-43	Aerated	2.53	3.00	3.66	5.18	3.59
	Anoxic	2.46	2.46	2.50	3.93	2.84
NG77-59	Aerated	2.96	3.14	4.91	7.18	4.55
	Anoxic	2.55	2.79	4.19	4.89	3.60
CP 65-357	Aerated	3.52	4.93	7.64	10.34	6.60
	Anoxic	3.47	4.60	6.45	8.49	5.75

[1/] Values are means of four plants.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 9. Root production by the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2/]	Root Dry wt. (mg plant^{-1})[1/]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
Cavengerie	Aerated	254	371	544	786	489
	Anoxic	215	241	260	273	247
D-74	Aerated	363	568	869	1082	720
	Anoxic	315	360	405	417	374
NG77-43	Aerated	325	505	746	1143	680
	Anoxic	282	340	360	362	336
NG77-59	Aerated	710	842	1060	1380	998
	Anoxic	579	595	649	792	654
CP 65-357	Aerated	529	790	2046	2644	1502
	Anoxic	484	560	1406	1644	1023

[1/] Values are means of four plants.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

DISCUSSION

The natural habitat of some Saccharum species is along river banks and intermitantly flooded areas (Berding and Koike, 1980; Clements, 1980) so that one would expect variable levels of flooding tolerance among the species of Saccharum.

The data presented here supports this with data showing considerable genetic variability in flood tolerance of different Saccharum species. The commercial hybrid (CP 65-357) and S. spontaneum (Coimbatore) were the most tolerant of the plants tested. They showed no adverse visual effects to anoxia and their root and shoot growth were reduced by only 31% - 46%. This supports previous reports by Stevens (1948) and, Sartoris and Belcher (1949), during the 1947 natural flood in Florida. They stated that the greatest potential for flooding tolerance lies in the species S. spontaneum. Panje (1971), in his study of the contribution of wild canes, considered S. spontaneum as the best donor of desirable characters among the extraneous parents used in interspecific hybridization with the noble cane. He further stressed that the primary objective of using S. spontaneum potential in a crossing program was for disease resistance, but the hybrids, besides being disease resistant, also inherited qualities to be well adapted to diverse agroclimatic conditions. The results obtained in the flooding test appear to be in good agreement with the above findings.

Stevens (1948) and Sartoris and Belcher (1949) suggested that primordial root production was important in the survival of some varieties. This is supported by the data in table 3 and 5. The more tolerant Coimbatore (S. spontaneum) and CP 65-357 (commercial hybrid) generally show greater promordial water root production, whereas Chunnee and NG 77-160 represented S. barberi and S. robustum respectively produce the least amount of primordial water roots and they are relatively intolerant under anoxic condition. An exception to this was found with D-74 (S. officinarum) where there was no correlation between the primordial root formation and tolerance to anoxia. According to visual symptoms and stalk elongation rate, this clone is intolerant. Yet its ability to produce primordial water root is much higher than that of the other intolerant clone NG 77-160 (S. robustum) and moderately intolerant Chunnee (S. barberi). Data from the second flooding-test (Table 3) indicate that, under flooded condition, D-74 depends on the main roots in soil (MR) as well as the primordial water roots (PR) to a considerable extent. The dependence of NG 77-160 and Chunnee on PR for stalk elongation or growth was, however, the maximum.

Primordial root production is one aspect of flooding tolerance in sugarcane which is related to the natural survival of sugarcane under flooded conditions, however, there are probably other anatomical or metabolic adaptations which occur due to varying periods of anoxia. The development of primordial water roots near the surface allows plants to grow under conditions where oxygen is not limiting. Growing plants in nutrient solution bubbled with air or nitrogen eliminates this aspect of tolerance and reveals a greater deviation in flooding

tolerance. The stalk elongation of 3 species ceased after 20 days of anoxia, although, the decrease in root production (47%) was not nearly as great for NG 77-160 as in Chunnee and D-74. This is in agreement with the reports that have accumulated in the literature. Kramer (1951) reported that tomato plants growing in flooded conditions had lower plant height than those under the control. Kramer and Jackson (1954) observed a cessation of stem elongation due to flooding in tobacco which is considered very sensitive to anoxia. In a similar case, Williamson (1970) reported that flooding and pure N_2 treatments for 24 hours almost prevented further growth in tobacco. After only 48 hours of anoxic treatment he observed that the roots and shoots were almost dead. Blake and Reid (1981), working on the flooding tolerance of three Eucalyptus species with variable tolerance, reported that in E. obliqua showing the greatest flooding damage, stem elongation slowed considerably after 7 days of flooding, and was reduced by 90% after two weeks of flooding. Stalk elongation of flood-tolerant E. camaldulensis was, however, least affected by flooding. Kennedy et al. (1980) reported a much reduced seedling fresh and dry weight of rice and barnyard grass under anaerobic conditions. Keeley (1979), working with three population types of Nyssa species with variable tolerance to flooding, reported that under flooding conditions all the three populations showed reduction in root/shoot ratio with the flood plain and swamp populations allocating much less to roots than shoots. Barclay and Crawford (1982) observed shoot growth of a number of wetland species under anoxia and reported that some species of Schoenoplectus, Scirpus, and Typha exhibited sustained shoot elongation under anoxia for 7 days, while some species of

Phragmites, Phalaris and Iris had no shoot elongation although they survived the anoxic treatment. Some species of Glyceria, Juncus, Ranunculus, and Cyperus were, however, killed by the anoxic treatment. Tang and Kozlowski (1982), working on flood-tolerant Betula pepyrifera, reported that flooding drastically inhibited the shoot growth and root growth, and caused decay of roots and death of many seedlings. Flooding also inhibited growth of leaves that formed prior to flooding, and stopped the formation of new leaves. They, however, found no evidence of any adaptive morphological changes to flooding.

Bull and Glasziou (1975), in a controlled experiment, observed reduced root growth of sugarcane cultivar 'Pindar' grown at high temperature. Nickel (1977) stated that sugarcane is a highly aerobic plant. In the nutrient culture with deficient aeration, he observed a decreased root mass production. Negi et al. (1971) reported that the sugarcane variety BO3 with superior tolerance to flooding, was conspiculously different from other varieties in the development of its root system. At 24 weeks of growth, the root system of this variety occupied the minimum area, but the highest density; at 36 weeks, however, it had the lowest number of roots.

Most of this present work is concerned with a comparison of flood tolerance of different Saccharum species. However, data on the varieties of S. officinarum indicate that there is variability among the different clones of this species. The stalk elongation of the clones Cavengerie, D-74, and NG 77-43 is severely inhibited, while NG 77-59 retained about 50% of its elongation ability during 30 days of anoxia. It is interesting that the decrease in stalk elongation was not mimicked by as large a decrease in shoot dry matter. For

example, D-74 which shows little stalk elongation has a greater increase in stalk dry weight during anoxia than all the other clones of S. officinarum tested (Table 8). All the species showed a greater increase in dry weight than stalk length during anoxia suggesting that part of the change in elongation is the result of a shift in growth pattern from an elongation mode to a change in width. This change in the pattern of growth could be due to the synthesis of plant hormone such as ethylene which is known to increase cell expansion vs. elongation. Also, ethylene increases in the shoot of some plants such as corn.

The method developed here for testing flooding tolerance in sugarcane reveal a range of differences in flood tolerance among the plants tested. This range of variability should be useful in sugarcane breeding or screening program and provide a framework for metabolic studies related to the mechanism of flooding tolerance in Saccharum species.

PART II. METABOLIC CHANGES IN SUGARCANE UNDER FLOODING OR ANOXIA

INTRODUCTION

Metabolic adaptations of plants to flooding have received considerable attention in the recent past. It has been recognized that besides having morphological and anatomical adaptations, plants tolerant to flooding are endowed with the ability to regulate their anaerobic metabolism. Chirkova (1978) stated that among the adaptations that enable plant to tolerate flooding or oxygen deficiency, physiological-biochemical adaptations representing a wide range of metabolic reactions regulated by the plant are significant.

Metabolic changes in response to flooding have been investigated in a large number of crop plants viz., rice (Vertapetian et al., 1978; John and Greenway, 1976; Bertani et al., 1980), wheat and barley (Pomeroy and Andrews, 1979; Wignarajah et al., 1976), maize (Ho and Scandalios, 1975; Marshall et al., 1973), pea (Smith and ap Rees, 1979a), sunflower (Torres and Diedenhofen, 1981), clover (Francis et al., 1974), alfalfa (Barta, 1980), and also in many trees and wetland species. The objective of these studies have mostly centered around understanding the biochemical mechanism of flooding tolerance with a view to develop suitable screening technique for flood tolerance.

Among the biochemical changes related to flooding, the activities of some enzymes viz., Alcohol dehydrogenase (ADH), Malate dehydrogenase (MDH), Pyruvate decarboxylase (PDC), and Lactate dehydrogenase have been mostly investigated. Considerable amounts of attention has

also been given to the end-products of anaerobic metabolism viz., ethanol, malate, etc.

So far, there has been no systematic metabolic studies on sugarcanes in relation to their flooding tolerance. The present investigation was undertaken to examine the changes in some enzyme activity and metabolites of interest in the roots of the different Saccharum species and a commercial hybrid with a view to identifying the metabolic responses that are related to flooding tolerance. The work was further extended to cover some S. officinarum clones in order to investigate intraspecific differences. The investigation included the enzymes ADH, MDH, PDC, and LDH, and the metabolites viz., ethanol, malate, sucrose, glucose, and fructose. In the experiment with S. officinarum clones, only three enzymes, ADH, MDH, and Peroxidase were investigated in addition to malate and ethanol.

LITERATURE REVIEW

The concept of metabolic adaptation of plants to flooding or anoxia is relatively new. Historically, however, the foundation-idea came into existence around 1860 when Louis Pasteur discovered that the rate of fermentation rises when oxygen is excluded. This phenomenon is customarily referred to as the "Pasteur Effect" - the term introduced by Warburg in 1926 (Phillips, 1947; Krebs, 1972). Intensive research in the 1930's led to the discovery of the sequence of reactions in the Embden-Meyerhof Pathway (Lehninger, 1975). This serves as the basic substratum of our present day understanding of metabolic adaptations by plants to flooding or anoxia.

In plants growing under an adequate supply of oxygen, the oxidation of carbohydrate yields energy for growth and metabolism, and normally takes place in three stages (Curtis, 1979; Lehninger, 1975). In the first stage or glycolysis, the conversion of 1 mol of glucose to 2 mol of pyruvate is accomplished by 2 mol of ATP and 2 mol of $NADH_2$. In the final stage of the oxidation of glucose, the energy stored in $NADH_2$ and $FADH_2$ is used in the synthesis of ATP in the mitochondrial respiratory chain. The electron and protons, transferred via the electron transport chain, is finally accepted by molecular oxygen giving water. Calculations show that the overall complete aerobic respiration of 1 mol of glucose yields 38 mol of ATP whereas anaerobic glycolysis delivers only 2 mol of ATP per mol of glucose (Curtis, 1979; Lehninger, 1975).

When plants are subjected to flooding, the depletion and resultant disappearance of molecular oxygen lead to the inactivation of Krebs cycle and cessation of activity of the cytochrome chain in the root cells (Fitter and Hay, 1981). This initiates anaerobic glycolysis or fermentation and builds up acetaldehyde along with the induction of the enzyme Alcohol dehydrogenase (ADH) which catalyzes the transformation of acetaldehyde to ethanol in the presence of $NADH_2$. In fact, the activity of this enzyme has been regarded as the corner-stone of the metabolic adaptation of plants to anoxia or flooding.

Fitter and Hay (1981) reported that the consequences of the oxygen deprived glycolytic pathway lead to two serious problems to the plants. (i) The end product of anaerobic fermentation, viz., acetaldehyde, ethanol, and lactate are potentially toxic and their accumulation in plant lead to cell disruption and eventually, death. (ii) Compared to the energy yield in aerobic respiration (38 ATP per mol of glucose), the energy yield in anaerobic pathway is very small (2 mol of ATP per mol of glucose). As a result, when anaerobic conditions are imposed, the rate of glycolysis must be enhanced sharply if the cells are to maintain energy status near to the aerobic level. This "Pasteur effect" may lead to rapid depletion of available carbohydrate with the concomitant build-up of toxic metabolites and eventual death of the root and shoot.

There are two predominant metabolic theories of plants' adaptation. Both theories involve the terminal stages of glycolysis. The first theory is based on the observations that under flooding or anoxia, some flood-tolerant plants exhibit accelerated alcoholic

fermentation. Response of this kind has been reported by Taylor (1942), John and Greenway (1976), Wignarajah et al. (1976), Rumpho and Kennedy (1981), Smith and ap Rees (1979a), Hook et al. (1971) and others. Keeley (1979) stated that accelerated alcoholic fermentation in response to anoxia is adaptive in nature, because, without it pyruvate and NADH would accumulate with the resultant blockade of glycolysis. Moreover, by using alcoholic fermentation, plants can get the advantage of glycolysis as the compensatory energy source in the absence of aerobic respiration. The second theory, mostly advanced by Crawford's group, argues that accelerated alcoholic fermentation is operative only in the intolerant plant species (Crawford, 1967; Fulton and Erickson, 1964; McManmon and Crawford, 1971; Crawford and Baines, 1977). On the other hand, plants tolerant of flooding avoid increased ethanol production by altering the route of carbon metabolism storing it as malate which is non-toxic (Crawford and Tyler, 1969; McManmon and Crawford, 1971). This theory is founded on the experimental evidences that under flooded condition intolerant species have higher rates of alcoholic fermentation than those in tolerant ones, and also that in the latter, the malate concentration is higher than in the intolerant, presumably as a means of avoiding ethanol toxicity. It deserves mention at this point that reports, both for and against the above two theories, have appeared in the literature (Francis et al., 1974; Wignarajah and Greenway, 1976; Bolton and Erickson, 1970; Marshall et al., 1973).

In one of his earlier work, Crawford (1967) reported that the Senecio species sensitive to flooding had higher Alcohol dehydrogenase (ADH) activity accompanied by higher ethanol concentration whereas

plants relatively tolerant to flooding showed no increase in ethanol concentration and no induction of ADH activity. In an attempt to explain the phenomenon that many flood-tolerant plants do not show Pasteur effect or accelerated fermentation under anoxia thereby avoiding depletion of carbohydrate and escaping the accumulation of toxic metabolites, Crawford (1978a) reported that ADH isoenzymes in flood-tolerant species have much higher Km values (ie. a much lower affinity for acetaldehyde, the substrate) than the ADH isoenzyme from flood-sensitive species. Working with nine flood-intolerant and ten flood-tolerant plant species, McManmon and Crawford (1971) observed that, after one month of experimental flooding in sand culture, the intolerant species showed very high level of ADH activity whereas the tolerant species did not show any such activity under flooding. They also reported the presence of malic enzyme in flood-intolerant species and absence from flood-tolerant species. In a separate study, Crawford and Tyler (1969) noted higher malate concentration in the roots of flood-tolerant species upon flooding. They concluded that the absence of malic enzyme provides a good reason for high malate accumulation in tolerant species while its presence in intolerant species serves as a route for the loss of malate via pyruvate, where it would presumably contribute further to ethanol accumulation. These evidences led to the advancement of McManmon and Crawford's metabolic theory presented in figures 4 and 5 for further clarification. Davies (1980) has provided a brief account on the salient features of this theory.

Flood-tolerant species (Figure 4), lacking malic enzyme, respond to anaerobic condition by producing ethanol and acetaldehyde, but

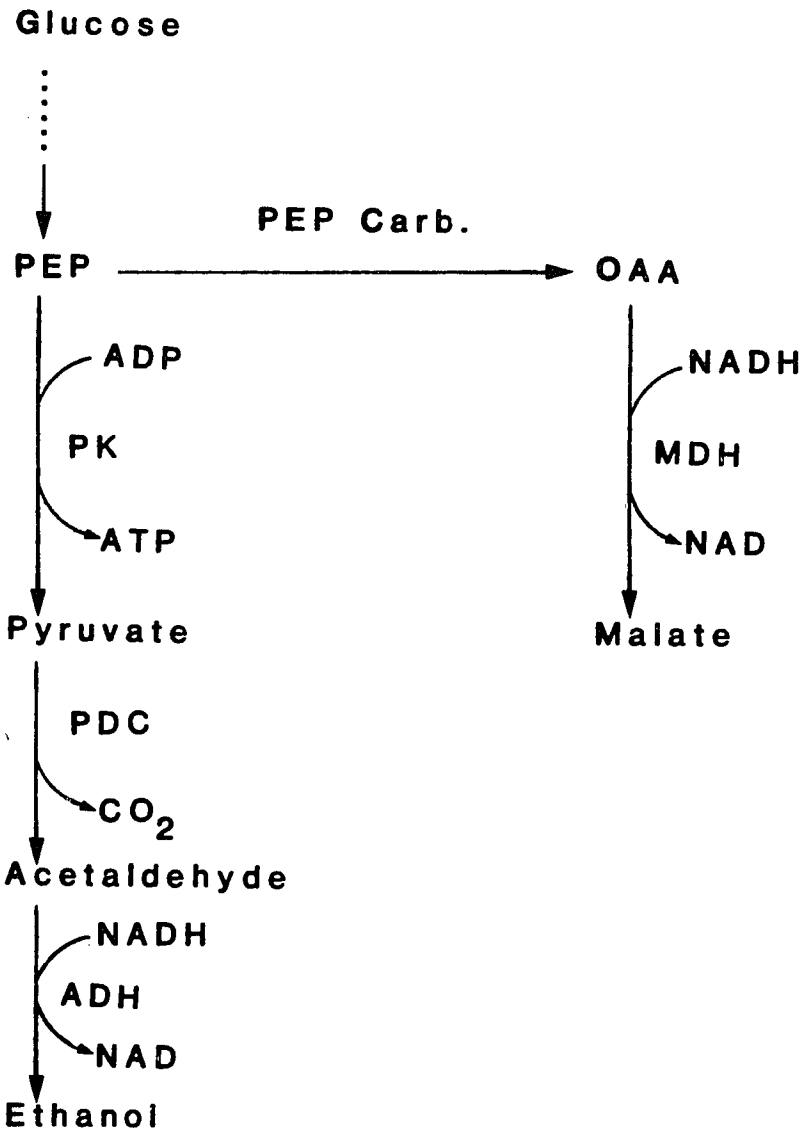

Figure 4. Metabolic scheme of events in flood-tolerant species upon flooding, characterized by the absence of malic enzyme, as proposed by McManmon and Crawford (1971). PEP=phosphoenolpyruvate, PEP Carb.=PEP carboxylase, OAA=oxaloacetate, MDH=malic dehydrogenase, PK=pyruvate kinase, PDC=pyruvate decarboxylase, ADH=alcohol dehydrogenase.

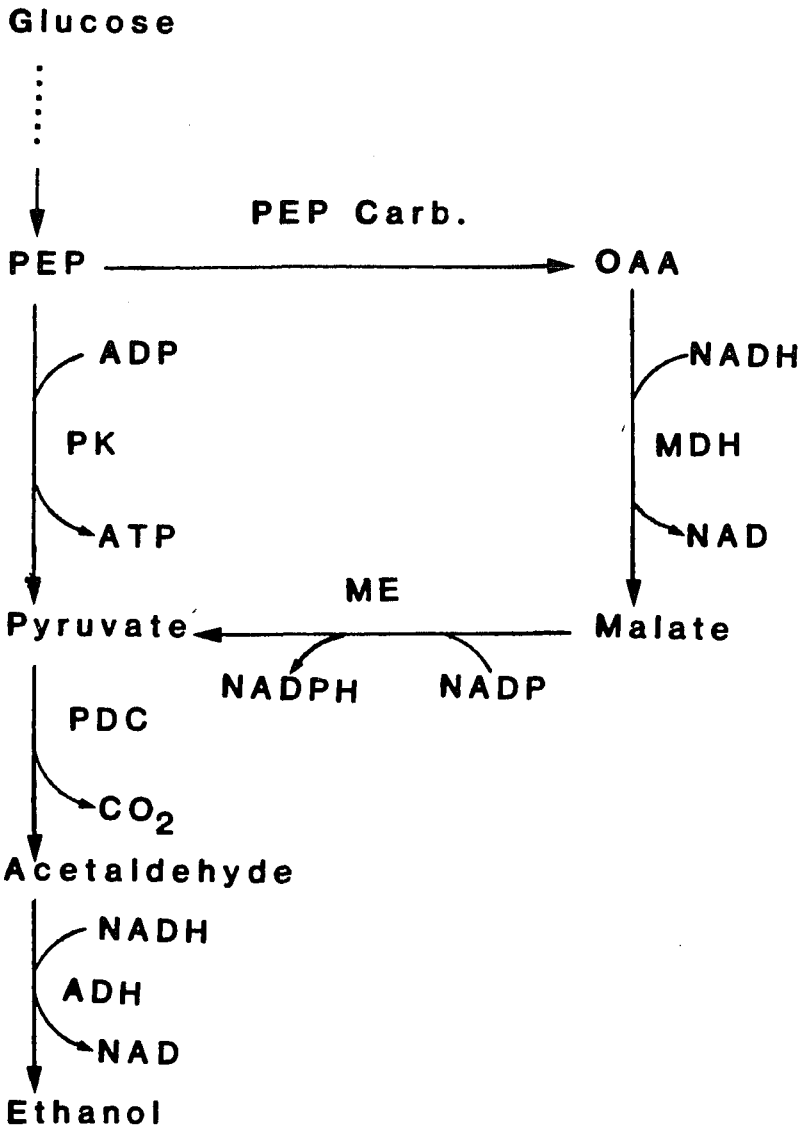

Figure 5. Metabolic scheme of events in flood-intolerant species upon flooding as proposed by McManmon and Crawford (1971). PEP=phosphoenolpyruvate, PEP Carb.=PEP carboxylase, OAA=oxaloacetate, MDH=malate dehydrogenase, ME=malic enzyme, PK=pyruvate kinase, PDC=pyruvate decarboxylase, ADH=alcohol dehydrogenase.

these products do not induce ADH activity and there is no accelerated glycolysis. Since malic enzyme is absent, malate is formed as an alternative to ethanol and accumulates. Flood intolerant plants (Figure 5), on the other hand, produce ethanol and acetaldehyde, which induces the ADH activity and accelerated glycolysis. Since malic enzyme is present, malate does not accumulate, but is decarboxylated to pyruvate and thence to acetaldehyde contributing further to ethanol production and resultant toxicity. Contrary to the report about the absence of malic enzyme from flood-tolerant species, Davies et al. (1974) demonstrated that malic enzyme was present in the roots of all four flood-tolerant species previously reported to lack this enzyme. Although this finding has weakened McManmon and Crawford's metabolic theory to some extent, this theory is still regarded as the foundation-work for further investigation to unveil the biochemical mechanism of flooding-tolerance in plants.

The present investigation was undertaken to determine the metabolic changes that might be involved in the adaptation of tolerant and intolerant sugarcanes (Saccharum spp.).

MATERIALS AND METHODS

Collection and Preparation of Root Sample

The growth of the plants used for this metabolic studies have been described in part I (vide: The Nutrient Culture Set-up). Roots were harvested 3, 10, 20 and 30 days after the initiation of anoxic treatment. Four plants of each species under both anoxic and aerated conditions were harvested at each harvest day. Harvested roots were quickly covered under ice flakes contained in buckets and brought to the cold room (5C) in the laboratory. Only the apical 4-5 cm root was sampled and wiped of excess water completely. A one gram sample was taken for enzymatic assays.

A separate root sample was taken for ethanol and malate determinations. The samples from each treatment were collected in pre-weighed 50 ml polypropylene centrifuge tubes (#03147, Sorvall Instrument Co.) and instantly frozen in liquid nitrogen onto which was layered 5 ml perchloric acid (8%) for deproteinization (Smith and ap Rees, 1979a). The weight of the roots used was determined by difference at later steps.

Extraction Procedures for Enzymatic Assays

Root samples were homogenized by grinding in a frozen mortar and pestle with an extraction buffer containing 50 mM HEPES, 2 mM Cysteine-HCl, and 5 mM $MgCl_2$ adjusted to pH 7.5 with KOH. A 10% (w/v) solution of PVPP (in the above buffer) and 2.75 g sand were added before homogenization. The extract was collected in polypropylene centrifuge tubes and centrifuged at 4C and 30,000 g for 25 min. The

supernatant was carefully transferred to 15 ml glass centrifuge tubes, volume recorded and kept in the refrigerator until assayed.

Enzyme Assays

All the enzymes, except Peroxidase (POD) were assayed by following the oxidation of pyridine nucleotide (NADH) at 340 nm and 25C using a double beam spectrophotometer (Perkin-Elmer 124) hooked to a recorder (Perkin-Elmer 56). The procedure and reaction mixture for each enzyme were as follows:

Alcohol Dehydrogenase, ADH (Alcohol:NAD oxidoreductase, EC 1.1.1.1) was assayed according to the procedure of John and Greenway (1976). The reaction mixture contained 40 mM HEPES, 5 mM $MgCl_2$, pH 8.0; 5 mM NADH; 10 mM acetaldehyde in a final volume of 3 ml. The reference cuvette contained everything except the substrate, acetaldehyde. The reaction was started by adding 200 µl of the substrate.

Malate Dehydrogenase, MDH (L-Malate:NAD oxidoreductase, EC 1.1.1.37) was assayed using the technique presented in the Worthington Manual (Anon., 1977) and Bergmeyer (1974). The reaction mixture contained 0.1 M phosphate buffer, pH 7.4; 2 mM NADH, and 10 mM OAA in a final volume of 3 ml. The reaction was started by adding 20 µl of the tissue extract.

Lactate Dehydrogenase, LDH (L-Lactate:NAD oxidoreductase, EC 1.1.1.27) was assayed with pyruvate as the substrate. The reaction mixture contained 45 mM MOPS, pH 7.0; 2 mM NADH, 22 mM pyuvate in a final volume of 3 ml. Reaction was started by adding 400 µl pyruvate, the substrate (Smith and ap Rees, 1979b; Bergmeyer, 1974).

Pyruvate Decarboxylase, PDC (2-oxo acid carboxylase, EC 4.1.1.1) was assayed by coupling the decarboxylation of pyruvate to the reduction

of acetaldehyde in the presence of commercial yeast alcohol dehydrogenase from Calbiochem (#12676). The reaction mixture contained 40 mM MES + 5 mM $MgCl_2$ (1:1), pH 6.3, 2 mM NADH, 0.5 mM TPP, 6 mM Pyruvate and ADH (0.01 g/ml) in a final volume of 3 ml. The reaction was started by adding 400 µl pyruvate, the substrate (John and Greenway, 1976; Bergmeyer, 1974).

Peroxidase (Donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) was assayed spectrophotometrically at 510 nm and at 25C according to the Worthington Manual (Anon., 1977). The reaction mixture contained 0.1 M sodium acetate buffer, pH 5.5, 0.0017 M hydrogen peroxide, the substrate, and 0.17 M phenol in 0.0025 M 4-amino antipyrine (proton donor) in a final volume of 2.95 ml. The reaction was started by adding 50 µl root extract.

Determinations on Metabolites

Ethanol and Malate

Root samples fixed in liquid nitrogen and deproteinized with 5 ml perchloric acid (8%) were kept in the freezer overnight. The frozen samples were thawed and their weight recorded. The root tissue was then homogenized for 30 sec with a polytron (Brinkmann Instruments Co.) at a speed setting of 5, and centrifuged at 30,000 g for 15 min. The supernatant was neutralized with 1.5 ml potassium carbonate (5 M) added dropwise and centrifuged at 30,000 g for 20 min. The supernatant was collected in 15 ml glass centrifuge tubes and volume recorded. This supernatant was used for the determination of both ethanol and malate enzymatically according to Bergmeyer (1974) using NAD and commercial preparations of Yeast Alcohol Dehydrogenase (#12676, Calbiochem) and malate dehydrogenase (#M-7383, Sigma). For

ethanol, the reaction mixture contained 75 mM Pyrophosphate buffer, pH 8.7, 75 mM Semicarbazide; 21 mM glycine; 24 mM B-NAD and 20 µl ADH (30 mg protein/ml) in a final volume of 3.32 ml.

The reaction mixture for malate contained 0.4 M Hydrazine, 0.5 M glycine, pH 9.0; 40 mM B-NAD and 10µl commercial MDH suspension (ca. 5 g protein/ml) in a final volume of 2.91 ml.

Sucrose, Glucose and Fructose were determined by a High Performance Liquid Chromatograph (HPLC) of Water Associates, Inc., Milford, MA. A 60 cm x 7 mm column packed with Aminex-Q1505 (K+) from Bio-Rad Laboratories combined with a 30 cm x 4 mm column packed with Synchropak ASC (ANSPEC Co.) was used.

RESULTS

A. Experiment with Saccharum species

Effect of Anoxia on Alcohol Dehydrogenase (ADH) Activity

The results on the effect of anoxia on root ADH activity of the different clones including the commercial hybrid are reported in table 10 and figures 6-9. In the intolerant clones D-74 and NG 77-160, there was a progressive increase in ADH activity in response to anoxic treatment (Figure 8). Comparison shows that NG 77-160 had a higher ADH activity after 3 days of anoxic treatments compared to D-74. After 30 days of anoxia, NG 77-160 had the highest level of ADH of any of the clones tested. The response of clones Coimbatore and CP 65-357, both found tolerant under anoxia, showed two different patterns (Figure 6). Coimbatore did not show an appreciable increase in ADH activity until 20 and 30 days of anoxic treatment. In CP 65-357, however, there was a high ADH activity (about 40 μ mol g^{-1} min^{-1}) detected at 3 days of anoxic treatment which gradually decreased over 30 days experimental period to almost the level of aerated control. The initial 3 days anoxic level of ADH was higher than any treatment with any of the clones except for the 30 days anoxic treatment of NG 77-160. Both Katha (moderately tolerant) and Chunnee (moderately intolerant) contained low levels of ADH throughout the experimental period, especially Katha (Figure 7). Chunnee had only a slight increase in ADH after 30 days of anoxia. In general,

Table 10. Alcohol dehydrogenase (ADH) activity in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2]	ADH Activity (μ mole NADH oxidized g^{-1} fresh wt. min^{-1})[1] Duration of Treatment (Days) 3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	0.7 (±0.0)	1.5 (±0.2)	1.8 (±0.4)	4.5 (±0.4)	2.1
	Anoxic	3.2 (±0.2)	3.3 (±0.8)	6.6 (±0.3)	13.6 (±0.4)	6.7
S. sinense (Katha)	Aerated	0.9 (±0.0)	0.9 (±0.0)	1.8 (±0.1)	1.8 (±0.5)	1.3
	Anoxic	1.8 (±0.2)	2.6 (±0.1)	5.6 (±0.2)	5.0 (±0.3)	3.8
S. barberi (Chunnee)	Aerated	1.2 (±0.1)	1.5 (±0.1)	1.7 (±0.1)	1.8 (±0.1)	1.5
	Anoxic	4.6 (±0.1)	10.1 (±0.4)	9.9 (±0.3)	3.0 (±0.1)	6.9
S. robustum (NG 77-160)	Aerated	1.4 (±0.1)	1.8 (±0.1)	2.4 (± 0.1)	4.6 (±0.3)	2.5
	Anoxic	17.2 (±0.1)	20.2 (±0.8)	20.0 (±0.3)	57.0 (±1.1)	28.6
S. officinarum (D-74)	Aerated	3.2 (±0.1)	2.3 (±0.1)	1.8 (±0.1)	2.1 (±0.2)	2.3
	Anoxic	8.7 (±0.1)	9.0 (±0.2)	20.4 (±1.7)	29.7 (±0.6)	17.0
Commercial Hybrid (CP 65-357)	Aerated	3.1 (±0.1)	2.2 (±0.1)	1.5 (±0.2)	2.1 (±0.1)	2.2
	Anoxic	40.5 (±0.1)	31.6 (±0.7)	11.1 (±0.8)	3.6 (±0.2)	21.7

[1] Values are means of four replicates ± standard error in parenthesis.

[2] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

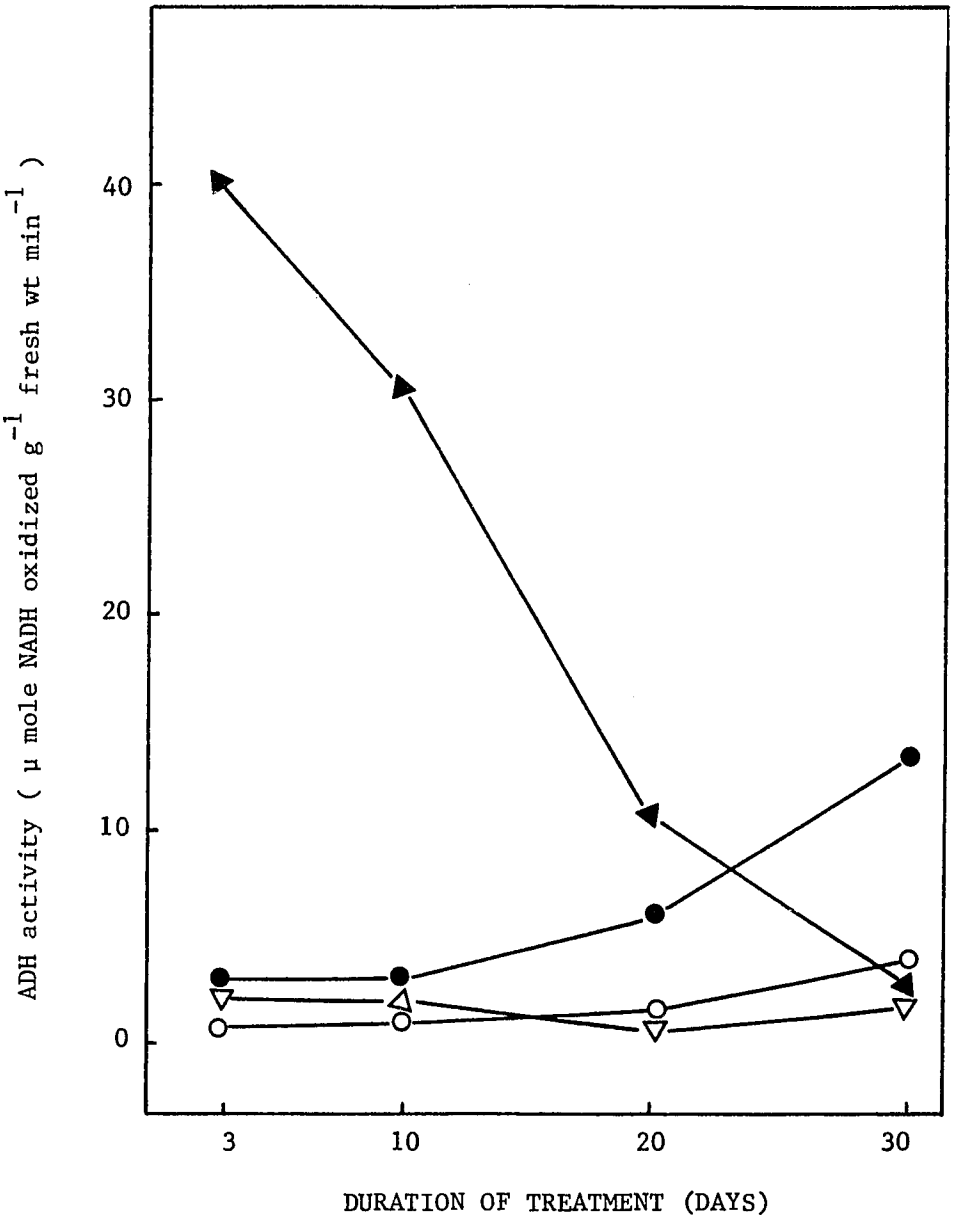

Figure 6. ADH activity in the root of clones Coimbatore (circle) and CP 65-357 (triangle) grown under aerated (open symbol) and anoxic (closed symbol) treatments. The largest standard error was 0.8 μ mole NADH oxidized g^{-1} fresh wt min^{-1}.

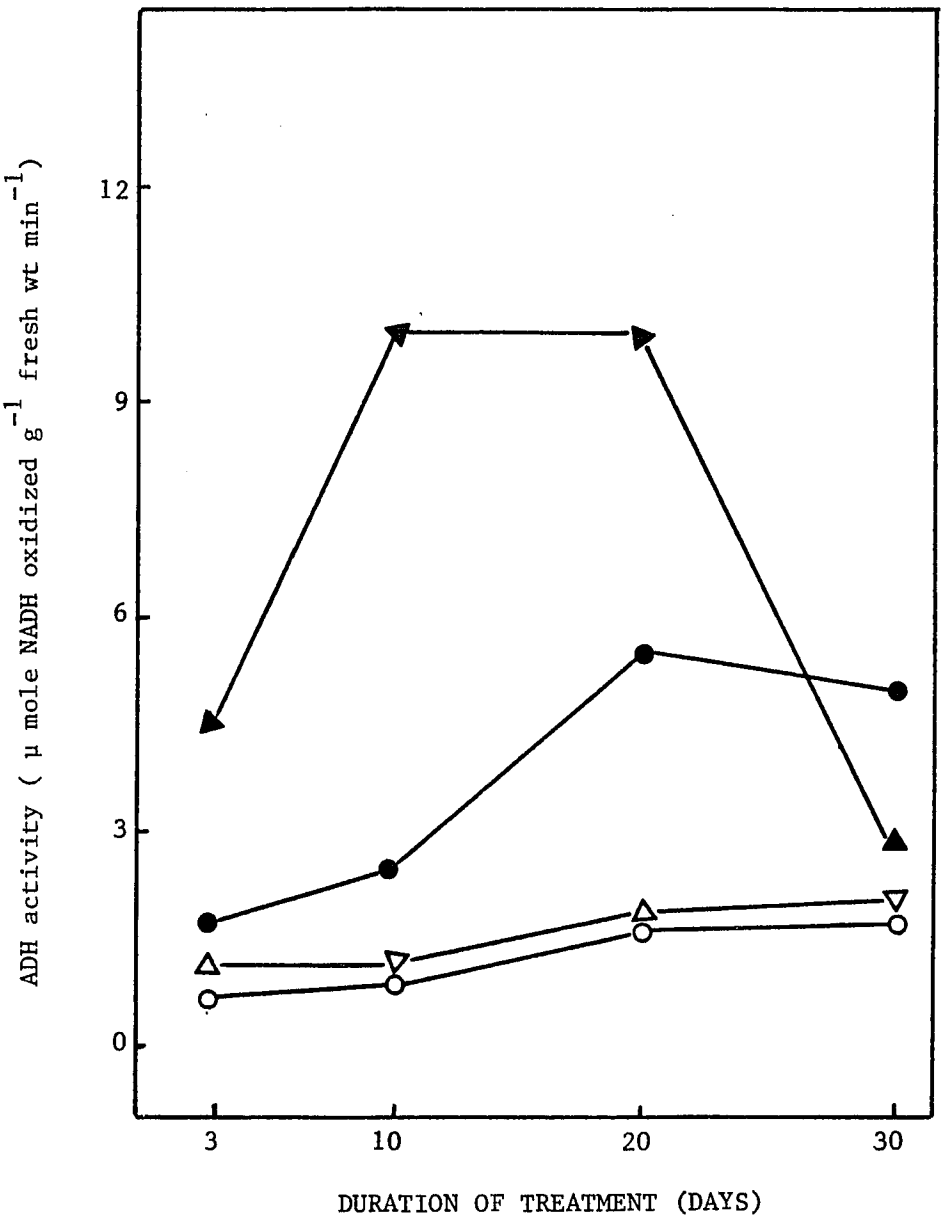

Figure 7. ADH activity in the root of clones Katha (circle) and Chunnee (triangle) grown under aerated (open symbol) and anoxic (closed symbol) treatments. The largest standard error was 0.5 μ mole NADH oxidized g^{-1} fresh wt min^{-1}.

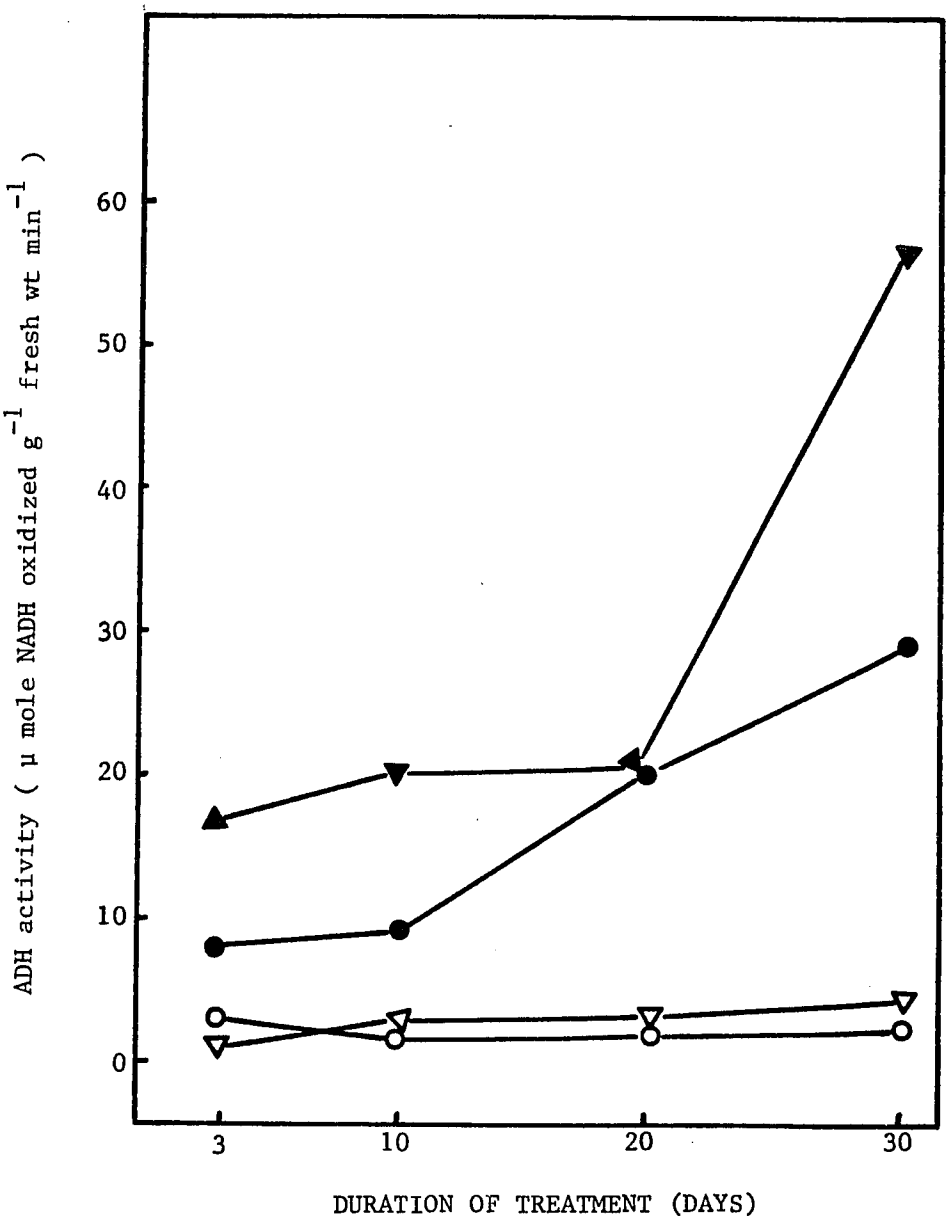

Figure 8. ADH activity in the root of clones D-74 (circle) and NG77-160 (triangle) grown under aerated (open symbol) and anoxic (closed symbol) treatments. The largest standard error was 1.7 μ mole NADH oxidized g^{-1} fresh wt min^{-1}.

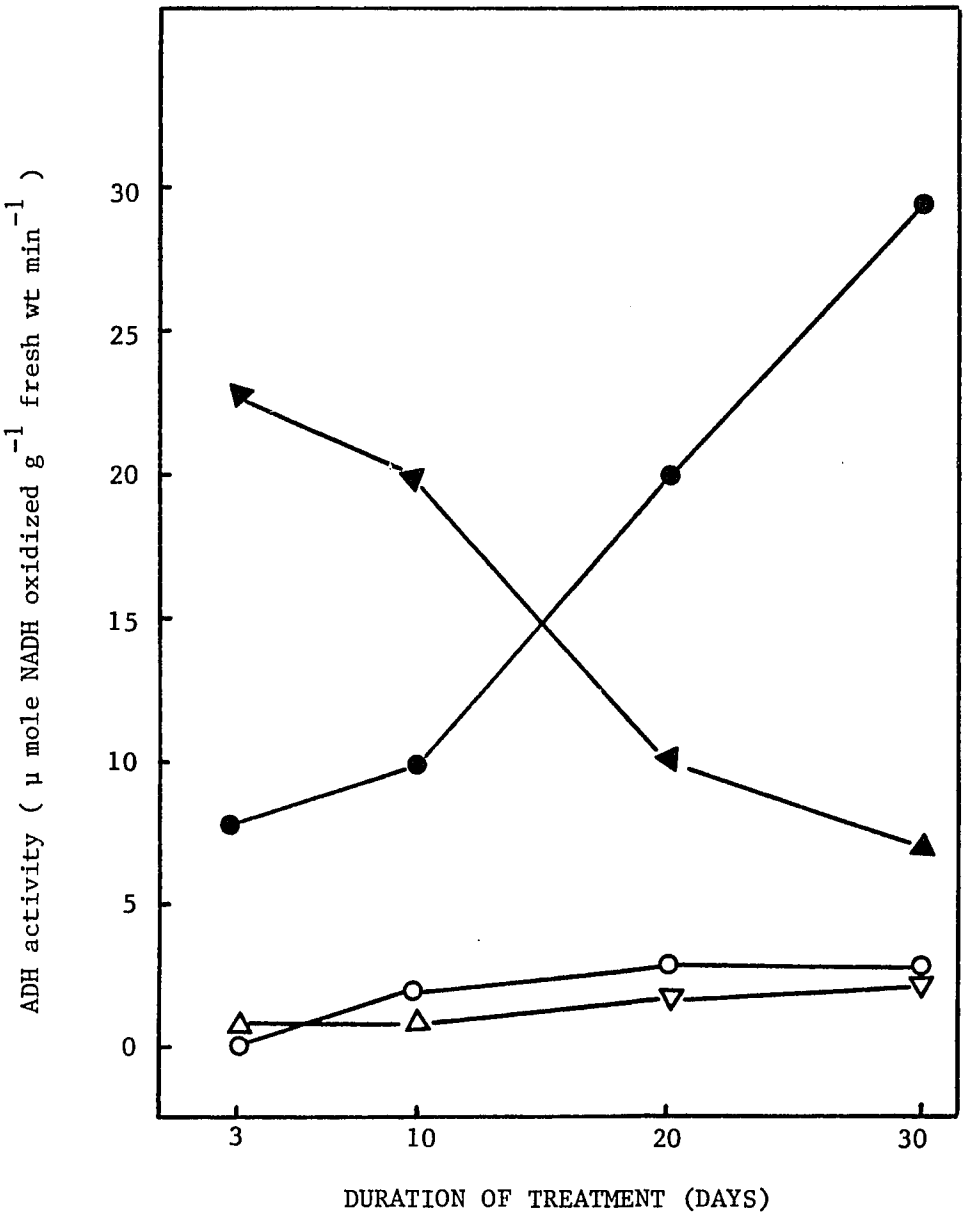

Figure 9. ADH activity in the root of clones Cavengerie (circle and NG77-59 (triangle) grown under aerated (open symbol) and anoxic (closed symbol) treatments. The largest standard error was 1.2 μ mole NADH oxidized g^{-1} fresh wt min^{-1}.

anoxia caused a small, but significant increase in ADH activity over the aerated control.

Effect of Anoxia on Malate Dehydrogenase (MDH) Activity

The MDH activity for the different clones grown under anoxic or aerated treatment is presented in Table 11. An average of MDH levels for tolerant clones (Coimbatore and CP 65-357) over 30 days of anoxia showed slightly lower levels of activity, whereas the intolerant clones (D-74, NG 77-160, Chunnee) had an average increase in MDH activity during the same period of anoxia. However, this response was not significant. No consistant trend in the activity of this enzyme was obtained except for the intolerant clone D-74 and NG 77-160, where the activity of the enzyme tended to drop gradually at least up to 20 days of anoxic treatment.

Effect of Anoxia on Pyrurate Decarboxylase (PDC) Activity

The PDC activity of the different clones grown under aerated and anoxic condition are presented in Table 12. There was no detactable PDC activity under aerated control. Under anoxic treatment, the activity was detectable only at 3 days of anoxic treatment. There was a significant difference in PDC activity among the clones. Clone Coimbatore (tolerant) had the highest level of response while D-74 (intolerant) had the lowest. The responses of clones Katha, Chunnee, NG 77-160, and the commercial hybrid, CP 65-357 were almost identical.

Effect of Anoxia on Lactate Dehydrogenase (LDH) Activity

Table 12 shows the LDH activity of the different clones. No detectable LDH activity was obtained either under anoxic or aerated

Table 11. Malate dehydrogenase (MDH) activity in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2]	MDH Activity (m mole g^{-1} fresh wt. min^{-1})[1] Duration of Treatment (Days) 3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	7.4 (±0.3)	2.7 (±0.3)	4.0 (±0.1)	6.1 (±0.2)	5.0
	Anoxic	4.2 (±0.4)	2.0 (±0.1)	5.7 (±0.3)	5.6 (±0.1)	4.3
S. sinense (Katha)	Aerated	4.0 (±0.2)	4.0 (±0.3)	2.9 (±0.2)	4.6 (±0.2)	3.9
	Anoxic	3.2 (±0.1)	2.3 (±0.3)	3.6 (±0.5)	4.2 (±0.1)	3.3
S. barberi (Chunnee)	Aerated	4.4 (±0.1)	3.3 (±0.1)	2.8 (±0.1)	4.3 (±0.1)	3.7
	Anoxic	4.2 (±0.4)	4.3 (±0.2)	4.0 (±0.3)	4.2 (±0.1)	4.2
S. robustum (NG77-160)	Aerated	6.8 (±0.2)	4.0 (±0.4)	4.0 (±0.1)	4.4 (±0.1)	4.8
	Anoxic	7.3 (±0.2)	5.5 (±0.3)	4.1 (±0.4)	5.3 (±0.4)	5.6
S. officinarum (D-74)	Aerated	3.7 (±0.1)	5.5 (±0.2)	3.8 (±0.1)	3.1 (±0.3)	4.0
	Anoxic	6.2 (±0.1)	5.1 (±0.1)	4.7 (±0.2)	4.6 (±0.2)	5.1
Commercial Hybrid (CP 65-357)	Aerated	4.0 (±0.1)	5.0 (±0.1)	5.8 (±0.2)	6.1 (±0.1)	5.1
	Anoxic	3.5 (±0.2)	4.9 (±0.2)	4.6 (±0.1)	4.9 (±0.2)	4.5

[1] Values are means of four replicates ± standard error in parenthesis.

[2] Statistical analysis showed no significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 12. Pyruvate decarboxylase (PDC) and lactate dehydrogenase (LDH) activities in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	PDC Activity[1/]		LDH Activity	
	Aerated	Anoxic	Aerated	Anoxic
S. spontaneum (Coimbatore)	NDA*	1.376	NDA	NDA
S. sinense (Katha)	"	0.685	"	"
S. barberi (Chunnee)	"	0.603	"	"
S. robustum (NG77-160)	"	0.627	"	"
S. officinarum (D-74)	"	0.482	"	"
Commercial Hybrid (CP 65-357)	"	0.656	"	"
LSD (0.05)		0.177		

* NDA = No detectable activity

[1/] Expressed as μ mole g^{-1} fresh wt. $min.^{-1}$, values are the means of three replicates. The activity was detectable only at 3 days.

conditions. Using commercial preparation of this enzyme as standard during the extraction it was found that exogenously added enzyme activity was not lost (unpublished result). This further indicated that if the enzyme were present, the levels of activity were too low to detect, and that the extraction and assay conditions for this enzyme were appropriate for the determination of its activity.

Effect of Anoxia on Ethanol Concentration

Table 13 shows the ethanol concentration in the root of the different clones grown under aerated and anoxic treatments. Anoxia significantly increased the ethanol concentration over the aerated control. Except for the commercial hybrid, CP 65-357, there was a progressive increase in root ethanol concentration in all the clones up to at least 20 days of anoxic treatment. In NG 77-160 (intolerant) and Chunnee (moderately intolerant), however, a drop in ethanol concentration was observed beyond 20 days. Plants under aerated control also had increases in ethanol concentration usually lower than 5 μM g^{-1} fresh weight of root to begin with. A distinctly different response was obtained with CP 65-357 which, under anoxia, exhibited a more than four-fold reduction in ethanol concentration over 30 days of anoxic treatment.

Effect of Anoxia on Malate Concentration

Table 14 shows the root malate concentration of the clones grown under anoxic and aerated treatments. Significant increases in malate concentration were observed under anoxic treatment of 10-30 days as compared to the control. Under anoxia, there was a general increase

Table 13. Ethanol concentration in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2]	Ethanol (μ mole g^{-1} fresh wt.)[1]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	2.4 (±0.7)	3.2 (±0.6)	8.4 (±0.5)	12.6 (±1.6)	6.7
	Anoxic	4.6 (±0.5)	8.1 (±1.3)	11.4 (±1.7)	12.9 (±1.4)	9.2
S. sinense (Katha)	Aerated	4.7 (±0.5)	2.8 (±0.2)	2.5 (±0.2)	8.4 (±0.6)	4.6
	Anoxic	6.7 (±0.7)	8.8 (±0.8)	10.9 (±0.8)	14.7 (±1.0)	10.3
S. barberi (Chunnee)	Aerated	3.6 (±0.2)	3.2 (±0.2)	3.3 (±0.1)	6.8 (±1.4)	4.2
	Anoxic	6.0 (±0.3)	13.3 (±1.9)	12.1 (±1.8)	8.7 (±0.7)	10.0
S. robustum (NG77-160)	Aerated	4.6 (±0.9)	6.5 (±1.5)	6.5 (±1.3)	2.9 (±0.4)	5.1
	Anoxic	4.7 (±1.1)	10.0 (±0.3)	17.9 (±1.6)	15.2 (±0.7)	12.0
S. officinarum (D-74)	Aerated	2.6 (±0.2)	5.8 (±1.4)	2.3 (±0.3)	8.4 (±1.0)	4.8
	Anoxic	7.4 (±1.5)	10.3 (±1.9)	14.6 (±2.1)	15.3 (±1.6)	11.9
Commercial Hybrid (CP 65-357)	Aerated	5.4 (±1.0)	5.7 (±1.3)	5.0 (±0.6)	2.3 (±0.2)	4.6
	Anoxic	16.5 (±2.5)	6.2 (±1.1)	6.6 (±0.9)	2.9 (±0.4)	8.0

[1] Values are means of three replicates ± standard error in parenthesis.

[2] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 14. Malate concentration in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2]	Malate (μ mole g^{-1} fresh wt.)[1]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	2.8 (±0.5)	1.4 (±0.3)	0.6 (±0.2)	1.0 (±0.1)	1.5
	Anoxic	2.3 (±0.1)	2.1 (±0.1)	6.1 (±1.0)	3.7 (±0.4)	3.6
S. sinense (Katha)	Aerated	0.5 (±0.1)	1.7 (±0.3)	1.6 (±0.2)	1.6 (±0.1)	1.4
	Anoxic	0.8 (±0.1)	2.0 (±0.3)	5.1 (±0.6)	3.4 (±0.5)	2.8
S. barberi (Chunnee)	Aerated	3.4 (±0.6)	1.1 (±0.1)	1.5 (±0.1)	0.9 (±0.1)	1.7
	Anoxic	1.5 (±0.1)	2.4 (±0.2)	3.1 (±0.4)	4.3 (±0.3)	2.8
S. robustum (NG77-160)	Aerated	1.2 (±0.1)	0.7 (±0.1)	1.7 (±0.2)	0.6 (±0.1)	1.0
	Anoxic	1.1 (±0.1)	3.3 (±0.5)	3.8 (±0.5)	6.0 (±0.6)	3.6
S. officinarum (D-74)	Aerated	1.4 (±0.2)	2.1 (±0.2)	0.8 (±0.1)	1.2 (±0.1)	1.4
	Anoxic	1.6 (±0.2)	2.7 (±0.3)	3.5 (±0.4)	4.4 (±0.5)	3.0
Commercial Hybrid (CP 65-357)	Aerated	0.6 (±0.1)	1.5 (±0.2)	1.2 (±0.1)	0.3 (±0.1)	0.9
	Anoxic	1.2 (±0.1)	1.6 (±0.2)	1.4 (±0.1)	3.8 (±0.5)	2.0

[1] Values are the means of three replicates ± standard error in parenthesis.

[2] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

in malate concentration in all the clones with the highest level attained during 20-30 days of anoxic treatment.

Effect of Anoxia on Sucrose Concentration

Table 15 shows the sucrose concentration in the root of the different clones grown under the aerated and anoxic treatments. Sucrose concentration was significantly higher under anoxic treatment than under the aerated control. Examinations of the anoxic data revealed that the average level of response varied among the different clones. D-74 (intolerant) followed by Coimbatore (tolerant) had the maximum concentration of sucrose per unit weight of root. The commercial hybrid, CP 65-357 (tolerant) had the lowest concentration of root sucrose under anoxic treatment.

Effect of Anoxia on Glucose Concentration

Anoxia significantly increased the glucose concentration over the aerated control (Table 16), there was however, no consistent trend of response over the experimental period. An examination of anoxic data reveals that the average level of glucose varied among the different clones. Chunnee, followed by Coimbatore, had the highest concentration of root glucose under anoxia, whereas Katha had the least.

Effect of Anoxia on Fructose Concentration

Table 17 shows the fructose concentration in the roots of the different clones subjected to anoxic and aerated treatments. The fructose concentrations were significantly higher during anoxic treatments than with the aerated control. Anoxic data revealed that the mean level of response varies among the different clones. The highest

Table 15. Sucrose concentration in the root of the Saccharum species and the commerical hybrid grown under aerated and anoxic conditions.

Species	Treatment[2/]	Sucrose (m mole g^{-1} fresh wt.)[1/]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	6	8	7	5	6.5
	Anoxic	40	22	27	26	28.7
S. sinense (Katha)	Aerated	7	5	7	11	7.5
	Anoxic	12	19	9	18	14.5
S. barberi (Chunnee)	Aerated	5	7	6	9	6.7
	Anoxic	18	44	14	8	21.0
S. robustum (NG77-160)	Aerated	8	5	5	6	6.0
	Anoxic	17	27	35	12	22.7
S. officinarum (D-74)	Aerated	6	4	5	6	5.2
	Anoxic	16	37	67	8	32.0
Commercial Hybrid (CP 65-357)	Aerated	7	4	6	7	6.0
	Anoxic	8	17	18	9	13.0

[1/] Values represent non-replicated composit samples.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 16. Glucose concentration in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2/]	Glucose (m mole g^{-1} fresh wt.)[1/] Duration of Treatment (Days) 3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	26	15	12	32	21.2
	Anoxic	66	41	13	132	63.0
S. sinense (Katha)	Aerated	23	10	24	23	20.0
	Anoxic	49	33	36	47	41.2
S. berberi (Chunnee)	Aerated	25	16	28	29	24.5
	Anoxic	61	96	25	27	52.2
S. robustum (NG77-160)	Aerated	30	9	15	16	17.5
	Anoxic	76	79	80	41	69.0
S. officinarum (D-74)	Aerated	30	16	12	26	21.0
	Anoxic	49	49	68	27	48.2
Commercial Hybrid (CP 65-357)	Aerated	11	12	8	41	18.0
	Anoxic	32	58	42	57	47.2

[1/] Values represent non-replicated composit samples.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 17. Fructose concentration in the root of the Saccharum species and the commercial hybrid grown under aerated and anoxic conditions.

Species	Treatment[2/]	Fructose (m mole g^{-1} fresh wt.)[1/]				
		Duration of Treatment (Days)				
		3	10	20	30	Mean
S. spontaneum (Coimbatore)	Aerated	23	4	102	54	45.7
	Anoxic	66	37	105	215	105.7
S. sinense (Katha)	Aerated	33	17	31	16	24.2
	Anoxic	44	158	9	78	72.2
S. barberi (Chunnee)	Aerated	41	11	34	39	31.2
	Anoxic	63	100	73	56	73.0
S. robustum (NG77-160)	Aerated	62	10	21	28	30.2
	Anoxic	108	78	86	54	81.5
S. officinarum (D-74)	Aerated	66	9	94	31	50.0
	Anoxic	94	45	79	41	64.7
Commercial Hybrid (CP 65-357)	Aerated	28	11	92	46	44.2
	Anoxic	49	72	70	62	63.2

[1/] Values represent non-replicated composit samples.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

concentration of fructose per unit weight of root was observed in Coimbatore, whereas the least was in CP 65-357 in spite of the fact that both the clones had superior tolerance to anoxia.

B. Experiment with S. officinarum Clones.

Effect of Anoxia on ADH Activity

Table 18 shows the root ADH activity of the different S. officinarum clones and commercial hybrid, CP 65-357 grown under aerated and anoxic conditions. There was a progressive increase in ADH activity in the intolerant clones viz., Cavengerie and D-74 and also in NG 77-43 (moderately intolerant) in response to anoxia. The tolerant clones NG 77-59 and CP 65-357 had high ADH level beginning the anoxic treatment but the level of response gradually declined with time, and by day 30, the activity dropped to almost one-fourth of the initial level (Figure 6 and 9). All the clones exhibited some amount of ADH activity detectable under the aerated control.

Effect of Anoxia on MDH Activity

Table 19 shows the MDH activity of the S. officinarum clones and the commercial hybrid, CP 65-357. Anoxia had no significant effect on the MDH activity over the aerated control. The trend of response was found to be inconsistent among the different clones over the experimental period. The tolerant clone NG 77-59 had higher MDH activity under anoxia, but the response of CP 65-357, also tolerant, was opposite. Similarly, D-74 (intolerant) had higher MDH activity under anoxia while intolerant Cavengerie had higher level of response under the aerated treatment.

Table 18. Alcohol dehydrogenase (ADH) activity in the root of the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2]	ADH Activity (μ mole NADH oxidized g^{-1} fresh wt. $min.^{-1}$)[1]				
		Duration of Treatment (days)				
		3	10	20	30	Mean
Cavengerie	Aerated	0.3 (±0.0)	2.1 (±0.1)	2.8 (±0.2)	3.1 (±0.6)	2.0
	Anoxic	8.3 (±0.2)	10.0 (±0.5)	19.9 (±0.8)	29.6 (±0.5)	16.9
D-74	Aerated	0.6 (±0.1)	1.0 (±0.2)	2.3 (±0.1)	3.2 (±0.1)	1.8
	Anoxic	15.7 (±1.8)	16.5 (±1.4)	29.3 (±1.5)	39.0 (±0.1)	25.1
NG77-43	Aerated	0.3 (±0.1)	0.3 (±0.0)	0.4 (±0.0)	0.9 (±0.1)	0.5
	Anoxic	8.3 (±0.1)	13.0 (±0.7)	36.8 (±0.8)	32.3 (±1.1)	22.6
NG77-59	Aerated	0.5 (±0.1)	0.8 (±0.0)	1.5 (±0.5)	2.7 (±0.2)	1.4
	Anoxic	23.6 (±1.2)	20.6 (±0.8)	10.7 (±0.9)	6.8 (±0.4)	15.4
CP 65-357	Aerated	0.5 (±0.1)	1.9 (±0.1)	4.1 (±0.7)	1.9 (±0.2)	2.1
	Anoxic	16.0 (±0.7)	16.8 (±0.7)	11.6 (±0.8)	4.3 (±0.0)	12.2

[1] Values are the means of two replicates ± standard error in parenthesis.

[2] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 19. Malate dehydrogenase (MDH) activity in the root of the Saccharum officinarum clones grown under aerated and anoxic conditions.

Clone	Treatment[2/]	MDH Activity (m mole g^{-1} fresh wt. $min.^{-1}$)[1/]				
		Duration of Treatment (days)				
		3	10	20	30	Mean
Cavengerie	Aerated	5.3 (±0.4)	9.4 (±0.3)	5.3 (±0.1)	7.7 (±0.1)	6.9
	Anoxic	5.8 (±0.2)	4.0 (±0.1)	4.8 (±0.4)	3.3 (±0.1)	4.5
D-74	Aerated	8.5 (±0.2)	8.6 (±0.1)	7.0 (±0.1)	9.8 (±0.3)	8.4
	Anoxic	10.9 (±0.3)	9.3 (±0.5)	9.1 (±0.1)	7.6 (±0.2)	9.2
NG77-43	Aerated	5.8 (±0.2)	5.6 (±0.1)	6.3 (±0.1)	8.1 (±0.3)	6.4
	Anoxic	7.8 (±0.7)	8.5 (±0.2)	8.5 (±0.1)	5.9 (±0.2)	7.7
NG77-59	Aerated	7.7 (±0.5)	7.5 (±0.3)	8.6 (±0.1)	8.6 (±0.1)	8.1
	Anoxic	9.7 (±0.5)	12.8 (±0.6)	13.0 (±0.2)	8.1 (±0.1)	10.9
CP 65-357	Aerated	5.8 (±0.2)	5.6 (±0.1)	6.3 (±0.1)	8.1 (±0.3)	6.4
	Anoxic	5.3 (±0.1)	8.0 (±0.2)	5.7 (±0.2)	6.0 (±0.1)	6.2

[1/] Values are the means of two replicates ± standard error in parenthesis.

[2/] Statistical analysis showed no significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Effect of Anoxia on Peroxidase (POD) Activity

The results on POD activity are reported in Table 20. There was no consistent difference in POD activities between the tolerant and intolerant clones. The mean response of the different clones varied considerably for both anoxic and aerated treatments. There was also a noticeable decline in POD activity at 10 and 20 days of the treatments.

Effect of Anoxia on Ethanol Concentration

Root ethanol concentration was significantly higher under the anoxic treatment than under the aerated control (Table 21). There was a progressive increase in ethanol concentration up to 20 days of anoxic treatments in clones Cavengerie and D-74 both found intolerant of flooding. The tolerant clones NG 77-59 and CP 65-357 showed a different response in which the ethanol concentration under anoxia decreased gradually up to at least 20 days. Under the aerated control, however, all the clones tended to accumulate higher concentration of ethanol with time (at least up to 20 days of treatment).

Effect of Anoxia on Malate Concentration

Root malate concentration was significantly higher under the anoxic treatment than under the aerated control (Table 22). No consistent trend in response was obtained in any clone during the experimental period. An examination of data, however, reveals that the mean level of response varied among the different clones. Under anoxia, clone NG 77-59 had the highest malate concentration while CP 65-357 had the lowest in spite of the fact that both the clones demonstrated superior tolerance to flooding or anoxia.

Table 20. Peroxidase (POD) activity in the root of the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2/]	POD Activity (ΔA_{510} g^{-1} fresh wt. $min.^{-1}$)[1/]				
		Duration of Treatment (days)				
		3	10	20	30	Mean
Cavengerie	Aerated	1622	640	660	1213	1034
	Anoxic	919	218	506	967	652
D-74	Aerated	2997	1086	323	1632	1510
	Anoxic	2545	812	733	3573	1916
NG77-43	Aerated	2035	907	785	2832	1640
	Anoxic	2093	739	518	2962	1578
NG77-59	Aerated	1308	892	224	2565	1247
	Anoxic	1549	535	542	2253	1220
CP 65-357	Aerated	860	185	95	324	366
	Anoxic	854	330	299	949	608

[1/] Values represent non-replicated samples.

[2/] Statistical analysis showed no significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 21. Ethanol concentration in the root of the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2]	Ethanol (μ mole g^{-1} fresh wt)[1]				
		Duration of Treatment (days)				
		3	10	20	30	Mean
Cavengerie	Aerated	2.0 (±0.3)	4.0 (±0.3)	4.9 (±0.2)	4.6 (±0.2)	3.9
	Anoxic	4.5 (±0.1)	4.9 (±0.1)	6.2 (±0.2)	6.6 (±0.5)	5.5
D-74	Aerated	1.9 (±0.1)	2.9 (±0.4)	4.4 (±0.2)	4.6 (±0.4)	3.4
	Anoxic	2.3 (±0.1)	5.3 (±0.4)	6.9 (±0.2)	4.3 (±0.2)	4.7
NG77-43	Aerated	0.9 (±0.1)	1.4 (±0.1)	1.7 (±0.1)	2.3 (±0.2)	1.6
	Anoxic	5.1 (±0.3)	4.9 (±0.3)	6.9 (±0.2)	4.7 (±0.2)	5.4
NG77-59	Aerated	1.3 (±0.2)	1.7 (±0.1)	4.5 (±0.2)	3.3 (±0.2)	2.7
	Anoxic	5.2 (±0.4)	6.1 (±0.3)	5.9 (±0.2)	4.5 (±0.2)	5.4
CP 65-357	Aerated	1.9 (±0.0)	3.1 (±0.4)	3.8 (±0.1)	2.0 (±0.1)	2.7
	Anoxic	7.2 (±1.1)	4.0 (±0.2)	4.9 (±0.4)	3.6 (±0.3)	4.9

[1] Values are the means of two replicates ± standard error in parenthesis.

[2] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

Table 22. Malate concentration in the root of the Saccharum officinarum clones and the commercial hybrid grown under aerated and anoxic conditions.

Clone	Treatment[2/]	Malate (μ mole g^{-1} fresh wt)[1/] Duration of Treatment (days) 3	10	20	30	Mean
Cavengerie	Aerated	1.1 (±0.2)	0.8 (±0.2)	1.3 (±0.1)	1.5 (±0.2)	1.2
	Anoxic	1.4 (±0.1)	1.4 (±0.1)	3.4 (±0.2)	2.8 (±0.1)	2.2
D-74	Aerated	0.8 (±0.0)	1.1 (±0.1)	1.3 (±0.1)	2.0 (±0.2)	1.3
	Anoxic	1.5 (±0.1)	2.9 (±0.0)	2.2 (±0.2)	3.3 (±0.1)	2.5
NG77-43	Aerated	0.7 (±0.1)	1.0 (±0.1)	0.6 (±0.1)	1.1 (±0.1)	0.8
	Anoxic	2.9 (±0.2)	2.9 (±0.0)	1.5 (±0.1)	1.6 (±0.1)	2.2
NG77-59	Aerated	0.6 (±0.1)	1.0 (±0.1)	1.3 (±0.1)	1.7 (±0.3)	1.2
	Anoxic	2.3 (±0.2)	3.6 (±0.2)	2.6 (±0.2)	3.0 (±0.0)	2.9
CP 65-357	Aerated	0.7 (±0.0)	0.6 (±0.1)	1.0 (±0.1)	1.1 (±0.1)	0.8
	Anoxic	1.6 (±0.1)	1.7 (±0.1)	1.2 (±0.1)	2.0 (±0.1)	1.6

[1/] Values are the means of two replicates ± standard error in parenthesis.

[2/] Statistical analysis yielded a significant difference between the anoxic and aerated treatments at $\alpha = 0.05$.

DISCUSSION

A progressive increase in ADH activity was observed in the intolerant clones, D-74, NG 77-160, Cavengerie and NG 77-43. On the other hand, the tolerant clones, viz., Coimbatore, CP 65-357 and NG 77-59 showed two different trends of response (Table 10, 18; Figure 6-9). The clone Coimbatore had a low level of ADH activity until 20 days of anoxic treatment, whereas CP 65-357 and NG 77-59 had high levels of ADH activity beginning the anoxic treatment which gradually decreased to the levels of aerated control over the 30 days experimental period. The response obtained with the intolerant clones appears to be in agreement with some reports accumulated in the literature. Crawford (1967) reported a high level of ADH activity accompanied by high ethanol concentration in the root of Senecio species sensitive to flooding. Plants relatively tolerant to flooding showed no such increase in ethanol production and no induction of ADH activity. Barta (1980) reported that following 7-days of flooding, alfalfa (Medicago sativa) exhibited severe injury symptoms accompanied by a rapid and high induction of ADH activity. Francis et al. (1974) reported a large (30-fold) increase in ADH activity, indicative of anaerobic respiration, in the roots of clover, Trifolium subterraneum, upon flooding. Crawford (1978b) mentioned that flood-tolerant species differ from intolerant species in not exhibiting an acceleration of glycolysis and show little or no induction of ADH when subjected to anoxia. McManmon and Crawford (1971) further strengthened this

concept that relative flood-tolerance was inversely proportional to ADH activity. Deviation from the above type of response has been reported by Smith and ap Rees (1979b). Working with three flood-tolerant species viz., Ranunculus sceleratus, Glyceria maxima, and Senecio aquatica, they observed enhanced ADH activity in these species in response to reduced aeration.

The differential responses of the tolerant clones in response to anoxia is not clearly understood. Some plausible explanation may however, be considered. Cytogenetically, there exists considerable difference among these tolerant clones. Clements (1980) mentioned the chromosome number of S. officinarum as 2n=80, while that of S. spontaneum is in the range of 2n=40-128. Specifically, the chromosome number of Coimbatore has been reported to be 2n=64 (Panje and Babu, 1960). Cossins (1978) reported that plant alcohol dehydrogenase exists as separate isoenzymes which could have differing substrate specificities. It is thus of some importance to identify possible changes in isoenzymes during induction by anoxia.

McManmon and Crawford (1971) reported some biochemical differences between flood-tolerant and flood-intolerant species. The ADH level of tolerant species was not induced by acetaldehyde, and therefore their tendency to accumulate ethanol would remain relatively small. On the other hand, the ADH of intolerant species was induced by acetaldehyde and displayed greater affinity for this oxidized substrate in response to flooding. This leads to the possibility that tolerant clones possess a particular ADH isoenzyme whose activity is induced by a relatively high level of ethanol, the reduced substrate. The relatively high levels of ethanol in Coimbatore detected at 20 and

30 days of anoxic treatment might have accounted for the increased ADH activity of this clone. The same explanation appears to hold true for the other two tolerant clones, NG 77-59, CP 65-357 (Table 10, 13, 18 and 21).

Pradet and Bomsel (1978) stated that two genes control the synthesis of ADH, Adh 1 and Adh 2. The balance of active gene products specified by Adh 1 and Adh 2 varies drastically in different tissue and in the same tissue in response to different environment such as anaerobiosis. Marshall et al. (1974) reported that in Lupinus (Lupinus angustifolius) with varying tolerance of flooding, the flood-tolerant strain contains only one band of ADH isoenzyme, whereas the flood-intolerant strain with high ADH activity, possesses two distinct ADH isoenzyme band.

The possible involvement of ethanol with concomitant increases in ADH activity has been reported by many researchers. In the present investigation, ethanol concentration of root was significantly higher in anoxic treatment than in the aerated control (Table 13 and 21). Except for the clone CP 65-357, there was a progressive increase in ethanol concentration in all the clones subjected to the anoxic treatment. Although ethanol has been extensively investigated in relation to flooding, its usefulness as a metabolic parameter is still doubtful (Chirkova, 1978; Cossins, 1978; Zemlianukhin and Ivanov, 1978; Hook and Scholtens, 1978; Mendelssohn et al., 1981; Keeley, 1979). Rumpho and Kennedy (1981) observed increased ethanol concentration in barnyard grass (E. crusgalli) subjected to anoxia. They noted that 85% of the ethanol produced by the seedling was in the external medium. Chirkova (1978) reported ethanol as the major toxic product of

anaerobic respiration causing cell disruption. Working with rice and wheat, he observed that the ethanol exudation into the external solution varies a great deal depending on the ecological nature of the plants. Crawford and Baines (1977) reported that in flood-intolerant Picea sitchensis, anoxia induced a twelve-fold increase in ethanol while in flood-tolerant Pinus contorta, the increase was only three-fold indicating a differential ability of species to limit ethanol production. Crawford (1978a) reported that ethanol accumulation resulting from increased glycolysis can readily lead to membrane destruction by lipid solubilization and this may inactivate mitochondrial enzyme activity. Similar observations were made by Herrero et al. (1982).

Keeley (1979) working with three population types of Nyssa sylvatica with variable tolerance to flooding, reported that the high level of root ethanol detected at the first week of flooding the upland plants (intolerant), was reduced by 80% at the end of one year of flooding. The relatively tolerant flood plain and swamp populations, however, continued to have increased level of ethanol beyond the first week, but after one year of flooding, the ethanol concentration was below that of the drained plants.

There was no significant difference in MDH activity between aerated and anoxic treatments. The data under the anoxic treatment, however, revealed that the mean level of response was a little higher in the intolerant clones viz., NG 77-160 and D-74. The metabolic significance of MDH in relation to flooding tolerance has not been clearly demonstrated probably because of its presence in the cytoplasm and other organelles (Fersht, 1977; Lehninger, 1975). Ugochukwu and

Anosike (1979) observed little or no MDH activity in yam tubers subjected to anaerobic conditions. Rumpho and Kennedy (1981), on the other hand, observed a two-fold increase of MDH activity in anaerobically grown barnyard grass (E. crus-galli). The high level of MDH activity in the tolerant clones Coimbatore and CP 65-357 (Table 11) under the aerated condition could be due to the utilization of malate via the TCA cycle reflecting the aerobic rate of the activity as suggested by Rumpho and Kennedy (1981).

A significant increase in root malate concentration was observed under the anoxic treatment as compared to the control. The intolerant clones showed a progressive increase in malate concentration whereas the relatively tolerant clones responded inconsistently (Table 14, 22). Although malate has received considerable attention in relation to flooding, its usefulness as a metabolic parameter is still questionable. Evidences for the accumulation of malate in the root of marsh plants under flooding were reported by Crawford and Tyler (1969) and McManmon and Crawford (1971) which led to the concept that flood-tolerant plants accumulate malate as an alternative end product to avoid ethanol toxicity (Figure 4 and 5). Keeley (1979) reported that malate concentration in the root of the upland population (flood-intolerant) of Nyssa sylvatica showed no significant difference between drained and flooded conditions. The highly flood-tolerant swamp population, after one month of flooding, had high malate concentration along with high ethanol and ADH levels. Crawford and Tyler (1969) also observed that malate content increased in helophytes (flood-tolerant) while it decreased in non-helophytes (flood-sensitive) during a four-day flooding experiment. Torres and

Diedenhofen (1981) reported that, in sunflower, malate concentration rose to high levels in the control plants, but remained low in flooded plants. Smith and ap Rees (1979b) observed no detectable accumulation of malate in the excised roots of three flood-tolerant species, viz., Ranunculus, Senecio, and Glyceria. Experiment with [U-^{14}C]-sucrose indicated a minimum labelling of malate, but appreciable labelling of ethanol under anaerobic condition. In another experiment, Smith and ap Rees (1979a) reported no accumulation of malate during a four-hour anoxic treatment of excised pea root. They, however, observed marked and continued accumulation of ethanol.

Pyruvate decarboxylase (PDC) activity was detectable only at 3 days of anoxic treatment in all the clones under study (Table 12). The level of activity was, however, very small compared to the other enzymes like ADH and MDH. The results obtained appear to be in agreement with others. John and Greenway (1976) reported that the PDC activity in anaerobically grown rice root was 15 times lower than the ADH activity in the same roots. They further mentioned that the PDC activity detected at the 10th day of anoxic treatment was less than 50% of what was detected at day 1 under the same conditions. Recently, Davies (1980) has stressed the significance of this enzyme in anaerobic metabolism by stating that the switch to ethanol production is essentially the activation of PDC by lowering the pH of the cytoplasm. Thus as fermentation proceeds, pH declines and induces the activity of PDC having an acid pH optimum. Concommitantly, the activity of Lactate dehydrogenase (LDH) which has an alkaline optimum, declines. The results obtained in the present investigation do not

support any metabolic significance of PDC under a relatively long-term flooding.

Lactate dehydrogenase (LDH) activity was not detectable under aerated or anoxic treatment. Similar results were also obtained by Rumpho and Kennedy (1981), and Ugochukwu and Anosike (1979). Smith and ap Rees (1979a), however, reported considerable amount of LDH activity in pea (Pisum sativum) grown under anaerobic conditions.

In another experiment, Smith and ap Rees (1979b) observed significantly higher LDH activity in Glyceria maxima and Senecio aquaticus under unaerated conditions. In Renunculus sceleratus, however, the response was not significant. Lambers (1976) also reported considerably high LDH activity in flood-tolerant Senecio species (S. aquaticus) grown under anaerobic conditions.

There was no significant difference in Peroxidase (POD) activity between the anoxic and aerated treatments. The mean responses of different clones varied considerably under both the treatments. The commercial hybrid, CP 65-357, showed the lowest level of response. POD has received some attention as an enzyme of interest in relation to flooding (Levitt, 1980; Chirkova et al., 1973; and Armstrong, 1967). Chirkova et al. (1973) reported an increase in POD activity in all the experimental plants belonging to two ecological groups (roots normally aerated, and, roots experiencing oxygen-deficiency) when subjected to anoxic treatment. However, this increase was greater at longer exposures in a nitrogen atmosphere in plants adapted to an oxygen deficiency. They mentioned that POD might enable plant to overcome the unfavorable effects of oxygen deficiency. Peroxides probably constitute the internal source of oxygen for tissue

respiration that compensates in some measure for a deficiency or absence of oxygen. It is likely that the lower level of POD activity in the tolerant clone CP 65-357 reflects its minimal dependence on this enzyme for compensating the oxygen debt by some mechanism.

There was a significant increase in sucrose, glucose, and fructose concentrations under anoxic treatment as compared to the control. The level of response considerably varied among the different clones regardless of their degree of tolerance to flooding. Cossin (1978), demonstrated that labelled ethanol was rapidly metabolized by a sequence that involved organic acids as the primary product, but with time, the percentage of C^{14}-incorporation declined in organic acids and rose in sugar.

Bertani et al. (1981) reported that in rice seedlings subjected to total anoxia, the concentration of reducing sugars and sucrose decreased immediately with the onset of oxygen-free environment. With the continuation of anaerobiosis, the amount of reducing sugars further decreased in contrast to the level of sucrose which showed a steady increase and regained initial values after 4 days. Starch concentration did not change significantly in absence of oxygen.

Barclay and Crawford (1983) reported root carbohydrate levels of three wetland species showing variable tolerance to anoxia, viz., Glyceria maxima (low tolerance), Phalaris arundinacea (medium tolerance), and Scirpus maritimus (high tolerance). They observed no significant changes in total non-structural carbohydrate (TNC) after four days of anoxic treatment in both S. maritimus and P. arundinacea in contrast to large decrease in TNC in G. maxima during the same treatment. They further reported that the levels of glucose and

sucrose did not significantly change in both S. maritimus and P. arundinacea. In the latter species however, they observed a four-fold increase in fructose concentration over 4 days of anoxia. All these species tested had large carbohydrate reserve as the stems were provided with rhizome. It is therefore, questionable as to whether this study should reflect the response of other plants like sugarcane which do not possess such structural modification.

SUMMARY

A significant variation in the ability of the different clones of Saccharum to produce primordial water root upon flooding was observed. The clone Coimbatore representing S. spontaneum followed by commercial hybrid CP 65-357 produced the highest quantities of water root. The clone Chunnee representing S. barberi and NG 77-160 representing S. robustum produced the least quantities of water roots. Under the pot-flooding test, none of the clones demonstrated severe symptoms of flooding injury. Presumably this was because of their ability to produce water roots within a short period of time. It was further observed that upon flooding the contribution by the water roots to stalk elongation growth was in general, more than that of the main roots in the soil (Table 3).

Under the anoxic nutrient culture, clones showed visual symptoms of injury, and were categorized as tolerant, moderately tolerent, moderately intolerant and intolerant, based on the severity of visual injury of the foliage. All the clones had significantly reduced rates of growth (stalk elongation) under anoxia as compared to the aerated control. The maximum reduction (97-98%) in stalk elongation was observed in the relatively intolerant clones viz., D-74, NG 77-160 and Chunnee (Table 5; Figure 1, 2). The tolerant clones viz., Coimbatore, and CP 65-357 suffered less reduction (46% and 41% respectively) in stalk elongation under anoxia. Intraspecific differences in growth rate under anoxia were observed. Among the clones of S. officinarum species, NG 77-59 showed a 53% reduction in stalk elongation and

proved to be tolerant to anoxia, whereas Cavengerie and D-74 had respectively, 80% and 98% reduction in stalk elongation under anoxia, and were shown to be intolerant. The moderately tolerant clone NG 77-43 had a 74% reduction in stalk elongation (Table 5; Figure 3). Intraspecific differences in shoot dry matter production under anoxia were observed with the clones of S. officinarum species but the response did not correlate with stalk elongation or anoxia-tolerance. There was an increasing trend of shoot dry matter production in all the clones even under anoxia.

Anoxia significantly reduced the root growth in all the clones tested as compared to the aerated control. The tolerant clones viz., Coimbatore and CP 65-357 demonstrated lesser reduction in root production under anoxia, compared to the relatively intolerant clones viz., D-74, NG 77-160, and Chunnee. Intraspecific differences in root production under anoxia was observed also with the clones of S. officinarum species. The tolerant clone NG 77-59 maintained a progressive increase in root production under anoxia, whereas, the relatively intolerant clones viz., Cavengerie, D-74, and NG 77-43 had greatly reduced root growth beyond 10 days of anoxic treatment.

Besides examining the growth and morphological parameters, considerable amount of attention was given to the metabolic parameters that could be useful in detecting the tolerant and intolerant clones and also understanding the mechanisms for such tolerance or intolerance. Intolerant clones viz., D-74 and NG 77-160 had a progressive increase in root ADH activity in response to anoxia. After 30 days of anoxia, NG 77-160 had the highest level of ADH of any of the clones tested. The tolerant clones viz., Coimbatore and CP 65-357

showed two different patterns of response. While CP 65-357 showed a high ADH activity beginning the anoxic treatment with a gradual decrease over 30 days of experimental period, Coimbatore did not show any appreciable increase in ADH activity until 20 and 30 days of anoxic treatment. Both Katha (moderately tolerant) and Chunnee (moderately intolerant) contained low level of ADH throughout the experimental period. Intraspecific differences in ADH level under anoxia were also detected with the clones of S. officinarum species. There was a progressive increase in ADH activity in the relatively intolerant clones viz., Cavengerie, D-74, and NG 77-43 in response to anoxia. The tolerant clone NG 77-59, however, had a higher ADH level beginning the anoxic treatment, but the level of response gradually declined with time, and by the day 30, the activity dropped to almost one-fourth of the initial level (Figure 9).

The mean levels of MDH activity in the tolerant clones viz., CP 65-357 and Coimbatore over 30 days of anoxia showed a slightly lower level of activity, whereas, the relatively intolerant clones D-74, NG 77-160 and Chunnee had an average increase in MDH activity under anoxia as compared to the aerated control. However, this response was not significant. No consistent trend in the activity of this enzyme was obtained except for the intolerant clones D-74 and NG 77-160, where the activity tended to drop gradually with time. In the S. officinarum clones, a similarly inconsistent trend was observed regardless of the degree of tolerance to anoxia exhibited by the different clones.

The activity of the enzyme PDC was detectable only at 3 days of anoxic treatment. There was a significant difference in PDC activity

among the different clones. The tolerant clone Coimbatore had the highest level of response while D-74 (intolerant) had the lowest. The responses of CP 65-357 (tolerant), and the other intolerant clones viz., NG 77-160, Chunnee were almost identical. LDH activity was not detectable either under anoxic or aerated conditions.

There was no consistent difference in POD activity between the tolerant and intolerant clones of S. officinarum. However, the mean response of the different clones varied considerably for both anoxic and aerated treatments.

Anoxia significantly increased the ethanol concentration over the aerated control. Except for the commercial hybrid, CP 65-357, there was a progressive increase in root ethanol concentration in all the clones upto at least 20 days of anoxic treatment. In the intolerant clones NG 77-160 and Chunnee, a drop in the ethanol concentration was observed beyond 20 days. A distinctly different response was obtained with CP 65-357 which exhibited a more than four-fold reduction in root ethanol concentration over 30 days of anoxic treatment. Intraspecific differences in root ethanol concentration in response to anoxia were observed with the clones of S. officinarum species. The intolerant clones Cavengerie and D-74 showed a progressive increase in ethanol concentration upto 20 days of anoxia, whereas, in the tolerant clone NG 77-59, the ethanol concentration gradually decreased with time.

The root malate concentration showed a general increase under anoxia in all the clones, but the trend of response was not very consistent in the different clones tested. Results with the S. officinarum clones also demonstrated a similar inconsistent response in both the tolerant and intolerant clones.

The concentrations of sucrose, glucose, and fructose in the root of the different clones increased in response to anoxia regardless of the degree of tolerance of the clones to anoxia. However, the average level of response varied among the different clones. D-74 (intolerant) followed by Coimbatore (tolerant) had the maximum concentration of sucrose per unit weight of root, while CP 65-357 (tolerant) had the lowest concentration of root sucrose under anoxia. The root glucose concentration under anoxia was the highest in Chunnee (moderately intolerant) followed by Coimbatore (tolerant) and the lowest in Katha (moderately tolerant). In the case of fructose, the highest concentration was obtained with Coimbatore and the lowest with CP 65-357 in spite of the fact that both the clones had superior tolerance to anoxia or flooding. The results of this investigation suggests that future research attentions be concentrated on the enzyme ADH, and the root and shoot growth characteristics under anoxia supplemented with translocation study and anatomical works.

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APPENDIX

Classification of the Saccharum clones used in the study

Clone	Classification	Reference
Coimbatore	S. spontaneum	Dunckelman and Breaux, 1971; Alexander, 1973
Katha*	S. sinense	Artschwager, 1954; Daniel et al. 1975; Barber, 1922; Price, 1957
Chunnee	S. barberi	Clements, 1980; Grassl, 1968; Stevenson, 1965
NG 77-160	S. robustum	Krishnamurthi and Koike, 1982
D-74	S. officinarum	Stevenson, 1965
Cavengerie	S. officinarum	Artschwager and Brandes, 1958
NG 77-43	S. officinarum	Krishnamurthi and Koike, 1982
NG 77-59	S. officinarum	Krishnamurthi and Koike, 1982
CP 65-357	Commercial Hybrid	Anon., 1973

* The classification of Katha has been disputed lately. Historically, Barber, in the early 1900's, made a detailed study of the Indian cane as S. sinense and divided them into five horticultural groups viz., Pansahi, Mungo, Nargori, Saretha and Sunnabile. These groups are also reported to have different chromosome number (Daniel et al., 1975). To the Saretha group belong the clones Chunnee and Katha (Artschwager, 1954). Jeswiet in 1925, revised the genus Saccharum and equated Panshahi group with S. sinense and transfered the remaining groups to a new species, S. barberi mainly on the

basis of thinner stalks and leaves. Artschwager (1954) followed by Price (1957) suggested that there was no basis for dividing the five horticultural groups into two species and included both under the original S. sinense. Recent chemotaxonomic studies indicated significant difference between S. sinense and S. barberi (Daniel et al., 1975). For the purpose of this dissertation, however, Katha has been retained as S. sinense and Chunnee as S. barberi. This is primarily because of the continued recognition of S. sinense as species (Clements, 1980) although some authorities are in favor of abolition of this speciation (Grassl, 1968).

VITA

A.B.M. Mafizur Rahman was born in the district of Jessore (Bangladesh) on March 13, 1947. After completing his high school education in 1963, he entered the Bangladesh Agricultural University where he received a B.Sc. (Ag.) degree in 1968. He held a US-NIH research assistantship through the Department of Soil Science, Bangladesh Agricultural University, for 1969 and received his M.Sc. (Ag.) degree of the 1969 academic year.

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The author is married to Rezia Begum. They have two daughters, Momi and Rimi.

EXAMINATION AND THESIS REPORT

Candidate: A.B.M. Mafizur Rahman

Major Field: Agronomy

Title of Thesis: Growth and Metabolic Responses of *Saccharum* Species in Relation to Flooding

Approved:

Fred A Martin
Major Professor and Chairman

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Date of Examination:

July 18, 1983